SEED SIZE, GROWTH AND FLOWERING STRATEGY IN ANNUAL PLANTS

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I DEDICATE THIS THESIS TO MY PARENTS DOMINIQUE AND PATRICK, MY BROTHER VINCENT, MY GRAND PARENTS AND MY FAMILY FOR THEIR ENCOURAGEMENT, THEIR SUPPORT, THEIR PATIENCE AND THEIR LOVE.

CONTENTS

CHAPTER 1

General introduction

"…plants are geological force of nature, one to be added to the pantheon of mighty forces traditionally thought to have moulded and recycled the Earth's landscape and climate throughout its 4.5 billion years." David Beerling (2007) *The emerald planet*

In contrast to many animals that can escape from environmental stresses, plants are unable to change location (Braam and Davis 1990). They have to adapt to the changes and stresses of their environment to survive. Despite these strong environmental pressures, large variation is observed in plants growing in the same environment. Growth strategies and life-history traits such as seed size vary impressively among species that share the same habitat (Harper et al. 1961, Janzen 1970, Grubb 1977). Seed size varies among species over a range of ten orders of magnitude from the dust-like seeds of *Orchidaceae* to 20 kg double coconuts of *Lodoicea maldivica* palms (Harper et al. 1970). In contrast, seed size within species is remarkably constant (Harper et al. 1970, Wulff 1986, Haig 1989, Silvertown 1989, Turnbull et al. 1999). The variation in seed size among species within ecological communities is greater than almost any other measurable features of coexisting plants (Salisbury 1974, Lord et al. 1995, Moles et al. 2005). What is the source of this variability? Which selection pressures led to such variation in traits and how is trait diversity maintained? In the context of a changing world, where habitats are perturbed, it is important to preserve diversity and investigate how species response to perturbation (Pimm 1984, Tilman and Downing 1994). The plants' plasticity and the plant's abilities to sense environmental change is therefore of extreme importance.

Many factors contribute to species coexistence and the maintenance of life-history diversity in plant communities (Dalling and Hubbell 2002). For example, competition/colonization trade-offs are probably important in maintaining diversity in forest communities (e.g. (Dalling and Hubbell 2002) and annual communities (Rees 1995, Turnbull et al. 1999, Coomes and Grubb 2003, Turnbull et al. 2004, Turnbull et al. 2008). To investigate the maintenance of trait diversity, model communities or model organisms are important. The annual communities are practical because of the shorter generation-time compared to trees. However, even these plants models require several years sometimes to

conduct experiments, especially multi-generational experiments. The annual *Arabidopsis thaliana* is the ideal plant organism to solve this time problem. It has a relatively short life cycle of about eight weeks (Alonso-Blanco and Koornneef 2000, Somerville and Koornneef 2002). Moreover, hundreds of ecotypes are available from stock centres. They exhibit a large variation in traits such as seed size, flowering time or plant height (Alonso-Blanco and Koornneef 2000, Somerville and Koornneef 2002, Shimizu and Purugganan 2005). *Arabidopsis* is self-fertilizing and reproduces rapidly. This enables to create artificial populations to investigate competition and strategies between species. The huge amount of information about *Arabidopsis thaliana* is a valuable resource to study diversity (Shimizu and Purugganan 2005).

Annual plants allow us to study maintenance of trait diversity in a community structured by competition-colonization trade-offs, where the possibility of species coexistence via a competition-colonisation trade-off has been investigated and shown (Turnbull et al. 1999, Turnbull et al. 2004, Turnbull et al. 2008).

One important element in the reproductive strategy of a plant is the partitioning of its seed output into many small seeds or a few large ones (Gadgil and Solbrig 1972, Smith and Fretwell 1974, Geritz et al. 1999). The model from Smith and Fretwell (Smith and Fretwell 1974) predicts that there will be a single optimum seed size that is evolutionarily stable (Lloyd 1987): individuals that produce seeds either smaller or greater than the optimum suffer reduced fitness (Lloyd 1987, Geritz 1995). However, this model fails to explain why such a variation of seed sizes is observed in nature between species that share the same habitat (Geritz 1995, Rees and Westoby 1997, Turnbull et al. 1999).

Small-seed species are associated with good colonising ability, i.e. enhanced dispersal (Rees 1995, Turnbull et al. 1999, Coomes and Grubb 2003, Turnbull et al. 2004), because if a seed size/number trade-off operates, small-seeded plants will produce more seeds (Smith and Fretwell 1974, Venable 1992, Turnbull et al. 1999, Nathan et al. 2002, Wender et al. 2005).

However, many plants are reported to produce large seeds despite the advantages of small seeds (Turnbull et al. 1999). The most common explanation of the existence of large seeded-species is their superior competitive ability (Rees 1995, Turnbull et al. 1999, Coomes and Grubb 2003, Turnbull et al. 2004). Large seed size confers an advantage of higher seedling survival or growth (Weis 1982, Stanton 1984, Weller 1985, Marshall et al. 1986), greater success in emerging from deep burial (Stanton 1984, Weller 1985, Wulff 1986, Mazer 1987) and positive effects on germination (Venable 1992). Thus large-seeded species may have greater competitive ability (Wulff 1986, Rees and Westoby 1997, Chacon et al. 1998, Turnbull et al. 1999, Coomes and Grubb 2003) and/or an establishment advantage (Freckleton and Watkinson 2001, Leishman 2001, Dalling and Hubbell 2002, Turnbull et al. 2004). In consequence, in more undisturbed landscapes large seeds may have an advantage because they are better competitors while in highly disturbed habitats small seeds may be selected because they are better colonizers.

In models, small and large seeds can both be maintained within a single habitat if one assumes extreme (i.e. infinite) asymmetric competition (Skellam 1951, Tilman and Downing 1994, Rees and Westoby 1997, Geritz et al. 1999). Asymmetric competition is an unequal sharing of resources as a consequence of larger individuals having a disproportionate competitive advantage over smaller ones (Freckleton and Watkinson 2001). In the case where asymmetry is infinite, a species with a particular seed mass would be totally unaffected by competition with any species with a lower seed mass, no matter how small the size difference (Kinzig et al. 1999, Levine and Rees 2002, Turnbull et al. 2008).Such infinite asymmetry is biologically unfeasible (Kinzig et al. 1999) and relaxing the assumption of extreme asymmetry only allows coexistence of a small number of species (Adler and Mosquera 2000). Another explanation of the maintenance of very different seed sizes would be an equalising trade-off between seed mass and seed number (Dalling and Hubbell 2002). Under size-symmetric competition, where resource capture is proportional to mass, the outcome of

competition could be insensitive to whether species produce many small seeds or fewer large ones. Seed mass would therefore be a neutral trait subject to genetic drift (Dalling and Hubbell 2002). However, in model simulation, this equalising trade-off has shown not to be neutral indicating that some other stabilising mechanism is also required (Turnbull et al. 2008).

CONCEPT AND OUTLINE OF THIS THESIS

We used a combination of experimental and modelling approaches to analyze these mechanisms with two annual systems: the genetic model plant *Arabidopsis thaliana* and a plant community formed from a pool of nine species of sand-dune annuals. These two systems allowed us to study coexistence of ecologically similar species by investigating traits involved in the competition-colonization trade-off as seed mass, seed output, flowering time and growth both intra-specifically (with *Arabidopsis*) and inter-specifically (with the sand dune annual community).

Our first system is the weedy annual plant *Arabidopsis thaliana* (L. Heynh.; family Brassicaceae) occupying rocky places and disturbed habitats such as the margins of agricultural fields (Shimizu and Purugganan 2005, Mitchell-Olds and Schmitt 2006). *Arabidopsis thaliana* (Figure 1) is a widespread annual native to Europe and central Asia and naturalized in North America.

Across this geographic range, it experiences a broad range of climatic conditions (Hoffmann 2002) and selective pressures. The phenotypic characterization of plants collected from different geographical regions revealed considerable genetic variation (Rédei 1970, Alonso-Blanco and Koornneef 2000). The wild lines collected, each genetically adapted to a particular habitat have been harvested in numerous places in the world constituting a unique collection. Inbred stocks are available for many natural *A. thaliana* accessions (ecotypes), originating across the species' range (Mitchell-Olds and Schmitt 2006).

Figure 1. Pictures of *Arabidopsis thaliana*: (a) the entire plant, (b) flowers, (c) a mature silique ready to disperse seeds and (d) siliques not mature on inflorescence stem. Photos (a) and (d) from the database AraPerox (A Database of Putative Proteins of Arabidopsis Peroxisomes), http://www.araperox.uni-goettingen.de/. Photo (b) from the Research Unit in Genomics and Bioinformatics, http://urgi.versailles.inra.fr/projects/GnpSNP. Photo (c) by C. Paul-Victor.

The model plant *Arabidopsis thaliana* allows us to exploit natural genetic variation in seed mass (Rédei 1970, Krannitz et al. 1991, Pigliucci 1998, Alonso-Blanco et al. 1999, Pigliucci 2002, Somerville and Koornneef 2002, Maloof 2003, Shimizu and Purugganan 2005). In order to maximise genetic variation we use a population of recombinant inbred lines (RILs; Alonso-Blanco and Koornneef 2000, Koornneef et al. 2004). To produce RILs, also called single-seed descent lines, two parent accessions are reciprocally crossed to produce an F2 generation (Figure 2). Individual members of the F2 generation are then self-pollinated

and propagated via single-seed descent until homozygosity is achieved (normally 8 generations; (Burr and Burr 1991). Once homozygosity has been reached, RI lines may be propagated indefinitely (Burr and Burr 1991). RILs can reveal phenotypes outside of the parental range of variation, thus maximising the range of phenotypic expression (Alonso-Blanco and Koornneef 2000).

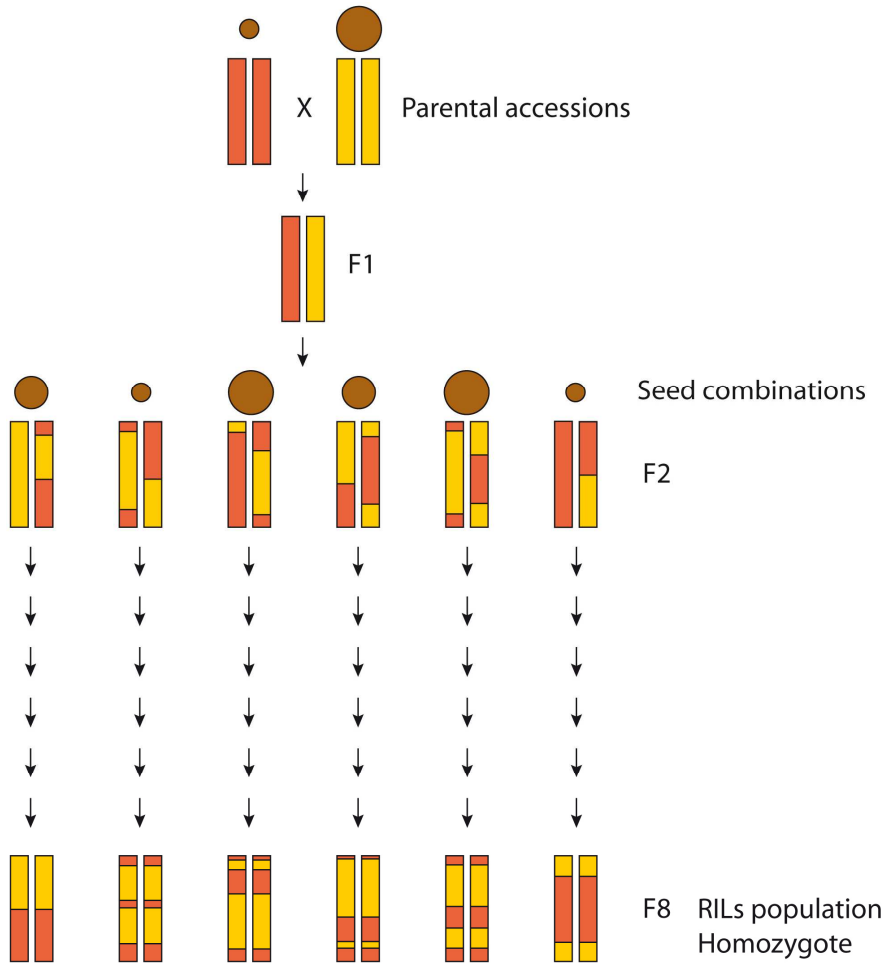

Figure 2. Production of recombinant inbred lines (RILs). Figure modified from Alonso-Blanco and Koornneef (2000). RILs are derived by successively selfing single plants from the progeny of individual F2 plants (single-seed descent method) until homozygosity is achieved at the F8 generation. Because they are homozygous, RIL populations can be permanently propagated and used indefinitely. The seed mass variation obtained from the two parental lines differing in their seed mass is illustrated by the brown seeds above the F2 generation.

We selected a set of RILs, polymorphic at eleven loci affecting seed size (Alonso-Blanco et al. 1999), derived from reciprocal crosses between the two pure lines Landsberg

erecta (L*er*), obtained as a mutant (*er*) from an accession of northern Europe (Rédei 1962, 1992), and Cvi, an accession from the tropical Cape Verde Islands (Lobin 1983). The two parents L*er* and Cvi have, respectively, small and large seeds (L*er*: 1.93 mg ± 0.10; Cvi: 3.51 mg ± 0.08; mass per 100 seeds, mean ± 1 SD; (Alonso-Blanco et al. 1999). The range in seed mass exhibited by the RIL population (1.45-3.73 mg per 100 seeds) is greater than the variation expressed by the two parents (Figure 3). The RILs selected from the possible set of 162 in our experiments are listed and described in more detail in each chapter.

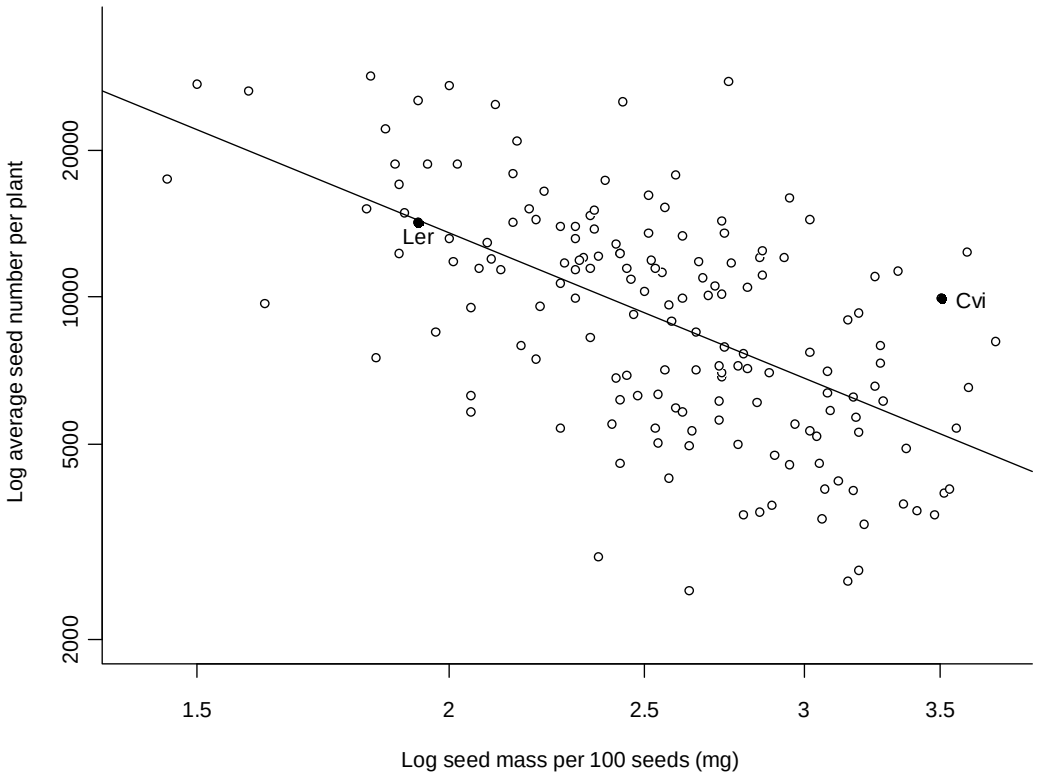

Figure 3. Relationship between estimated seed number per plant and seed mass for 100 seeds with the data from Alonso-Blanco et al. (1999). The two parental lines Ler and Cvi are pointed out with black circles. The white circles represent the 162 RILs derived from the two parents. The fitted line represents the significant negative relationship (slope = -1.68) from a linear model between seed number and seed mass ($F_{1,160} = 84.4$, $p < 0.0001$).

The lines can inherit the mutation *erecta* from the L*er* parent. Lines carrying the *erecta* mutation typically have round leaves, short petioles and pedicels. The flowers are clustered at

the top of the inflorescence and the siliques are short and wide with blunt tips. Stems are short and upright with a compact inflorescence and reduced height (phenotype curated by ABRC on TAIR). The *erecta* mutation has also been shown to reduce relative growth rate (RGR) over a 15 day growth period (Mitchell-Olds 1996).

Our second system consists of nine common annual sand-dune species (Figure 4). These species are commonly found throughout Europe and co-occur in sand dunes and gravel areas. They germinate in September and usually finish flowering by the end of June. All the species have been used in preliminary work and have been intensively studied (Mack and Harper 1977, Rees 1995, Turnbull et al. 2004, Turnbull et al. 2007). Seed weights of all species are given in Table 1. The nine annuals present a range of seed masses which span nearly three orders of magnitude.

Table 1. The nine sand-dune species and their seed masses (which span nearly three orders of magnitude).

Species	Seed mass (g)
Saxifraga tridactylites	0.006
Erophila verna	0.025
Cerastium semi-decandrum	0.045
Arenaria serpyllifolia	0.088
Veronica arvensis	0.112
Myosotis ramosissima	0.213
Valerianella locusta	0.851
Geranium molle	1.094
Erodium cicutarium	2.924

In a field study, these annuals exhibited a perfect seed size/number trade-off with a slope of -1 on log-log axes (Figure 5) which is consistent with a simple reciprocal relationship between seed number and seed mass (Turnbull et al. 1999, Coomes and Grubb 2003). This implies that on average individual has roughly the same amount of resources to allocate to reproduction regardless of species identity, and that species producing larger seeds therefore suffer reduced fecundity by having less seeds (Turnbull et al. 1999).

Figure 4. Photos of the nine species of the sand dune annuals, (a) *Erodium cicutarium*, (b) *Myosotis ramosissima*, (c) *Valerianella locusta*, (d) *Veronica arvensis*, (e) *Cerastium semi-decandrum*, (f) *Saxifraga tridactylites*, (g) *Erophila verna*, (h) *Geranium molle* and (i) *Arenaria serpyllifolia.*

Photo (a) from Southwest Colorado Wildflowers, ferns and trees, http://www.swcoloradowildflowers.com. Photo (b) from Botanischer Garten Ruhr-Universität Bochum, Germany, http://www.ruhr-uni-bochum.de/boga/. Photo (c) from Johnson's Creek website, http://www.johnsonscreek.co.uk. Photo (d) from Josef Hlasek, http://www.hlasek.com. Photo (e) from Fotografien von Wildpflanzen, http://www.flogaus-faust.de. Photo (f) from The Flora of Derbyshire, http://www.derby.gov.uk/dccwebdev/museum/flora/. Photo (g) from Odezia atrata, http://www.odezia-atrata.be/Flora/Brassicaceae/Erophila-verna/141-Erophila-verna.htm. Photo (h) from flowering and non-flowering plants of Missouri, USA, http://www.missouriplants.com/. Photo (i) from Erick Dronnet, http://erick.dronnet.free.fr/belles_fleurs_de_france/.

These nine annual species show competitive abilities linked to seed size (Mack and Harper 1977, Rees 1995, Coomes et al. 2002, Turnbull et al. 2004). It was shown that, within this community of annuals, the larger-seeded species produce fewer seeds on an individual basis and it was demonstrated by a sowing experiment that they are more strongly recruitment limited (Turnbull et al. 1999). Large or large-seeded species, therefore not only have an establishment advantage (Turnbull et al. 1999), but a true competitive advantage, and the greater the size differential the greater the competitive differential between species.

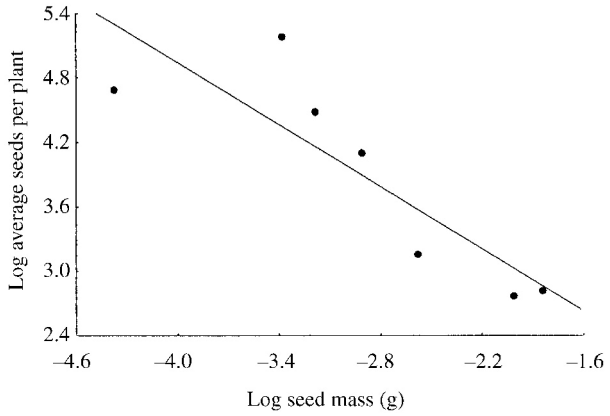

Figure 5. Average per capita seed production (calculated over 2 years) plotted against mean seed mass (log scales) from (Turnbull et al. 1999). The fitted line is $y = 1.096 - 0.961x$ (r^2 = 0.74).

The competition/colonization trade-off appears to operate within this group of annuals, and almost certainly acts as an equalizing mechanism, even if it is not sufficient alone to explain coexistence (Turnbull et al. 2004). The seed size/number trade-off can be a critical determinant of relative abundance patterns, provided some other mechanism maintains diversity (Levine and Rees 2002). Therefore seed size is a key trait in this group of species. It has been shown by Turnbull et al. (2004) that seed mass influences both competitive and colonizing ability. The presence of such competition/colonization trade-off undoubtedly stabilizes community dynamics although other mechanisms may also be at work.

The main aim of this study is to understand coexistence of ecologically similar species by investigating traits involved in the competition-colonization trade-off as seed mass, seed output, flowering time and growth both intra-species (with *Arabidopsis*) and inter-species (with the sand dune annual community).

Chapter 2 investigates the effects of changing the nutrient status of the environment on the nature of the seed size/number trade-off. We used naturally occurring variation in *Arabidopsis thaliana* with 32 RILs showing a large seed size range. We also focus on the difficulties to see the seed size/number trade-off because empirical evidence for this trade-off in plants is limited and contentious leading some to question the utility of this concept.

Chapter 3 focuses on the bolting decision in annual plants (the decision to switch from the vegetative to the reproductive phase) and considers whether plants make the switch at an optimal time. We used naturally occurring variation in *Arabidopsis thaliana* with the same set of RILs used in chapter 2. We also aim to understand how sensitive and plastic is the flowering switch for *Arabidopsis thaliana* and if the optimal solution involves a strict strategy (an age rule with a rigid, highly constrained flowering time) or the ability to make tactical responses (the plants would have the ability to "sense" from environmental cues and change their flowering time in the short term).

Chapter 4 explores the flowering strategy in *Arabidopsis thaliana* but in the context of different landscape dynamics. We employ a subset of the RILs previously used in chapter 2 and 3 to investigate how landscapes characteristics influence plant morphological traits associated with dispersal ability through a multi-generational experiment. We measure the effects of five generation of selection on these plants traits by growing them under standardized conditions. We are particularly interested to analyse the effects on traits thought to be associated with dispersal and competitive ability as height, *erecta* mutation seed size and seed number.

Chapter 5 examines the relative growth rate (RGR) concept commonly used to measure and compare species' intrinsic growth potential. Previous studies revealed that small-seeded species have higher RGR, leading to the common belief that small-seeded species grow faster, which would allow them to outgrow large-seeded species, given sufficient time. Because RGR declines as plants grow, RGR is heavily biased by initial plant size. As the outcome of competition in the long term is determined by such results, it is therefore highly important to measure the size-corrected growth potential. We investigate the growth of the nine coexisting annual species showing a large range of seed sizes by developing a daily growth model. The large seed size range combined with multiple harvests allows us to investigate the relationship between seed mass (initial size) and intrinsic growth rates.

The chapters 2, 3 and 4 are written as independent manuscripts for publication. Therefore there is inevitably overlap between some sections about the material description for *Arabidopsis thaliana* lines description and growth conditions, and overlap between sections explaining seed size/number trade-offs background and the introduction.

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CHAPTER 2

Now you see it; now you don't. The elusive seed size/seed number trade-off.

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ABSTRACT

Empirical evidence for seed size/seed number trade-offs in plants is limited and contentious leading some to question the utility of this concept. We show that even within a homogeneous environment, the seed size/number trade-off might not be seen among individuals, but rather among populations. We begin by showing that whether the trade-off occurs among individuals or populations depends on the relationship between seed size and adult size; and that this in turn depends on the nature of growth. Only when adult size is constrained by the environment, is a perfect trade-off among individuals expected. Here, we present the results of an experiment exploiting natural genetic variation in the model plant *Arabidopsis thaliana*. We used a selection of recombinant inbred lines (RILs) derived from reciprocal crosses between the small-seeded Landsberg *erecta* (L*er*) and the large-seeded Cape Verde Islands (Cvi). In an experiment using two pot sizes we show that adult size is only a function of pot size and not of seed size and that a perfect individual-level trade-off emerges between sown seed mass and seed number. Lines produced smaller seeds in smaller pots, but this plasticity was much less pronounced in lines carrying the *erecta* mutation.

INTRODUCTION

If individuals achieve the same average adult size, but choose to produce offspring of different sizes, then a trade-off will emerge between the number and the size of the offspring produced (Smith & Fretwell 1974). This leads to an expected relationship between seed size and seed number with a slope of -1 when plotted on log-log axes. However, empirical evidence for such a trade-off in plants is limited and contentious (Mazer 1987; Michaels et al. 1988; Winn 1988) leading some to question the utility of this concept (Michaels et al. 1988; Moles & Westoby 2006) and see Rees & Venable (2007). Clearly, the trade-off can be masked if comparisons are made among species from different communities with different typical adult sizes or productivity. Under these circumstances, individuals in good locations can potentially produce both more and bigger seeds (Maddox & Antonovics 1983; Venable 1992); however we show that even within a homogeneous environment, the seed size/number trade-off might not be seen among individuals, but rather among populations. We begin by showing that whether the trade-off occurs among individuals or populations depends on the relationship between seed size and adult size; and that this in turn depends on the nature of growth. To avoid complications that might arise as a result of differences in lifespan we restrict ourselves to a discussion of annual plants which differ only in seed size (i.e., species and genotypes are used here interchangeably). We then report the results of a controlled experiment that conforms as closely to this as possible: inbred lines of *Arabidopsis thaliana* that differ in their seed sizes grown under identical conditions.

Case 1: logistic growth

When plants are grown in pots, their individual growth curves can often be well-described by a logistic curve (Hunt 1982). For example, in the simplest case (a 2-parameter logistic curve) the

expected mass of an isolated individual of species or genotype *i* after time *t* in the absence of competition is:

$$M_{i,t} = \frac{K_i S_i \exp(\alpha_i t)}{K_i + S_i(\exp(\alpha_i t) - 1)}, \qquad \text{(eqn 1)}$$

where K_i is the maximum adult size that species or genotype *i* can attain, S_i is the seed size (i.e. the initial plant size) and α_i is the intrinsic growth rate. If two genotypes with different seed sizes can achieve the same adult size, *K*, then given sufficient time they should both achieve this mass regardless of differences in their intrinsic growth rates, α_i. This uncoupling of adult size from seed size is particularly likely to occur when isolated plants are grown in pots because adult size is determined primarily by the resources available in the pot. The number of seeds which a plant can produce is then $n_i = \varepsilon K / S_i$ (eqn 2) where ε is the conversion efficiency of final mass into seeds (also assumed to be equal for all species or genotypes). Equation 2 produces the seed size/number trade-off because any increase in S_i must result in a decrease in n_i (Smith & Fretwell 1974). Notice that there is no relationship between seed size and adult size although smaller seeds should take longer to reach the common adult size, *K*. If there is a constant mortality risk per unit time, this leads to a single optimum seed size, as other things being equal, individuals from smaller seeds are exposed to a longer period of risk (Kiflawi 2006).

Case 2: exponential growth

However, we could rather assume that plants grow for a fixed time interval, either because plants follow age-dependant rules (Silvertown 1983; Klinkhamer et al. 1990; Rees et al. 1999; Childs et al. 2003) or because the environment only allows a limited number of days for growth. In this case, isolated plants might achieve exponential growth in the absence of competition (as has been apparently observed for desert annuals; Angert et al. 2007). They

therefore achieve an adult mass at time *t*, $M_{i,t}$ given by $M_{i,t} = S_i \exp(\alpha_i t)$ (eqn 3). If we assume that the growth period, *t*, is the same for all species or genotypes, and that all individuals have the same intrinsic growth rate, α, then if two species *i* and *j* begin from different seed mass, the ratio of their final mass is given by $M_{i,t} / M_{j,t} = S_i / S_j$ (eqn 4). Thus, if genotype *i* has twice the seed mass of genotype *j*, it will also have twice the final mass and thus can produce the same number of seeds: $n_i = n_j = n$ (eqn 5) as $M_{i,t} / S_i = M_{j,t} / S_j$ (from eqn 4). Thus, in this case the trade-off between seed size and seed number seems to have disappeared.

In fact, under these circumstances the trade-off could still appear; but at the level of the population rather than the individual. To see this we need to further assume that the environment only provides *R* belowground resources per unit area and that the availability of these resources limits growth. Each individual must then exploit an area of ground (A_i) proportional to its adult mass in order to obtain sufficient resources to achieve this mass: $A_i = M_i / \mu R$ where μ is the conversion efficiency of belowground resources into plant tissue. However, if $A_i \propto M_i$, then from eqn 4, $A_i \propto S_i$. Thus, although each individual produces the same number of seeds regardless of its seed size (eqn 5), the area required to do so is proportionally greater for large-seeded individuals and so the seed output per unit area of ground is greater for small-seeded species. Thus the trade-off still operates but not among individuals: large-seeded species produce fewer seeds per unit area instead of per adult plant (Henery & Westoby 2001; Moles et al. 2004). Unlike the case of logistic growth, if there is a constant mortality risk per unit time, all seed sizes have equal fitness, because the period of risk, *t* is the same for all individuals (Kiflawi 2006). Thus the stage is potentially set for seed size to be a neutral trait (Hubbell 2001, Cadotte 2007, Schamp et al. 2008, Turnbull et al. 2008b).

Experimental evidence

To test whether these ideas apply in reality, we present the results of an experiment exploiting natural genetic variation in the model plant *Arabidopsis thaliana* (Krannitz et al. 1991; Maloof 2003; Shimizu & Purugganan 2005; Somerville & Koornneef 2002). Plants were grown alone in pots of two different sizes to mimic different degrees of belowground growth restriction within a realistic range. We grew individuals of 32 genotypes with widely varying seed sizes from a population of Recombinant Inbred Lines (RILs; Alonso-Blanco et al. 1999). We found in both pot sizes that after a fixed period of growth, seed mass and final mass were uncorrelated, and that as expected there was a perfect trade-off between seed mass and seed number among individuals.

MATERIAL AND METHODS

Plant material

To maximise genetic variation we used a population of RILs from Alonso-Blanco et al. (1999). To produce RILs, also called single-seed descent lines, two parent accessions are reciprocally crossed. Individual members of the F2 generation are then self-pollinated and propagated via single-seed descent until homozygosity is achieved (normally 8 generations; Burr & Burr 1991). Once homozygosity has been attained, RILs may be propagated indefinitely (Burr & Burr 1991). RILs can reveal phenotypes outside of the parental range of variation, thus maximising the range of phenotypic expression (Alonso-Blanco & Koornneef 2000).

We selected a set of RILs derived from reciprocal crosses between the two pure lines Landsberg *erecta* (L*er*), obtained as a mutant (*er*) from an accession of northern Europe (Rédei 1962; Rédei 1992), and Cvi, an accession from the tropical Cape Verde Islands (Lobin 1983). The two parents L*er* and Cvi have, respectively, small and large seeds (L*er*: 1.93 mg ± 0.10; Cvi: 3.51 mg ± 0.08; mass per 100 seeds, mean ± 1 SD; Alonso-Blanco et al. 1999). The range in mean seed mass exhibited by the original lines described in Alonso-Blanco et al. (1999) is 1.45-3.73 mg/100 seeds and is greater than the variation expressed by the two parents. We

selected 30 RILs from the possible set of 162, plus the two parent lines, for the experiment described here. The 30 lines were selected by dividing the original 162 lines (Alonso-Blanco et al. 1999) into six equally-spaced seed mass groups. We then selected five lines at random from each seed mass group in order to obtain a balanced distribution across the seed mass range. The lines can inherit the mutation *erecta* from the L*er* parent. Half of the selected lines carry this mutation, the other half not (Table S1 in appendix). Lines carrying the *erecta* mutation typically have round leaves, short petioles and pedicels, a short and upright stem, a compact inflorescence and reduced height (phenotype curated by the Arabidopsis Biological Resource Centre (ABRC)). The *erecta* mutation has also been shown to reduce relative growth rate (RGR) over a 15-day growth period (Mitchell-Olds 1996).

Experimental design

The seeds were obtained from The Arabidopsis Information Resource (TAIR) and we weighed a single sample of 100 seeds from each of the 32 selected lines (range: 1.286-4.107 mg/100 seeds). This is referred to as sown seed mass and was used as an explanatory variable in all analyses. All seeds were then placed in a cold room at 4 °C for one week to synchronise germination. All lines were grown in both small cylinders (10 mm diameter) and large cylinders (40 mm diameter) inserted into standardised cells (65 mm diameter) within a flat completely filled with compost. Each flat contained 35 cells and was 70 mm deep. The cylinders allowed us to randomise pot diameter treatments within flats and ensured that the spacing of individuals in different pot sizes and the surface area available to growing rosettes was exactly the same (Figure 1). Rosettes from neighbouring cells were never observed to overlap. However, the two pot sizes provide different degrees of belowground growth restriction. This enabled us to see whether adult size was genetically or environmentally-determined and whether adult size was a function of seed size in either environment. We aimed

to have five replicates of each line and pot size combination in a blocked design but due to germination failures the final design was slightly unbalanced.

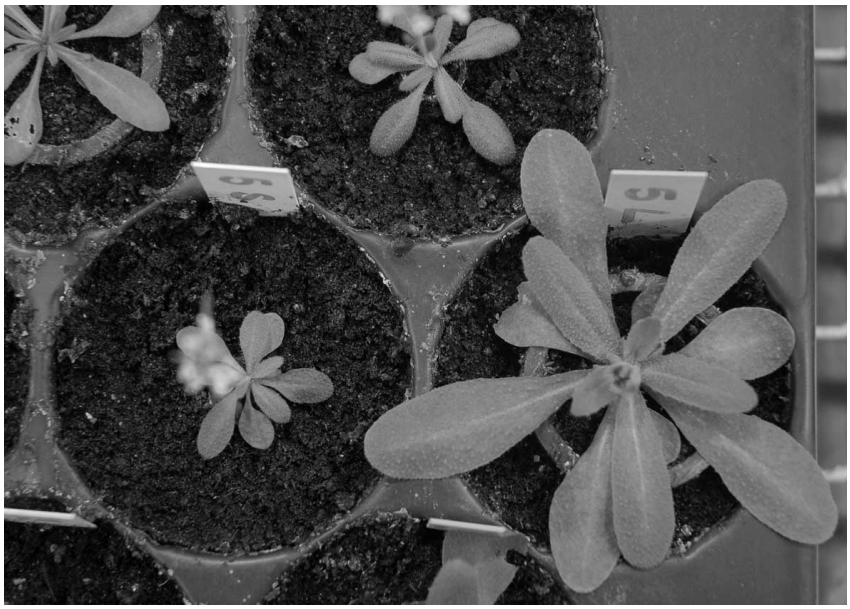

Figure 1. Picture of the experiment showing 10 and 40 mm diameter cylinders inserted into cells within a single flat. The two plants shown are genetically identical (from the same line). Note that the surface area available to growing rosettes is exactly the same for both treatments.

Pots were sown with four seeds and thinned as soon as seedlings emerged to leave one plant per pot (the most central healthy seedling). The plants were grown in a glasshouse with both natural light and additional artificial lighting when the natural light was below 25 kLux and kept under a cycle of 16 h light (22 °C) and 8 h dark (20 °C). When the plants began to produce fruits, we put perforated bags around the inflorescence to collect all the seeds produced by each plant. We continued watering until we observed complete senescence of plants, including all plant parts: 78 days in total. Thus all plants were allowed to grow until they naturally senesced, which should maximise their seed production in a given environment. After 78 days, all seeds from each plant were weighed to give the total mass of seeds. In order to estimate the total number of seeds produced by each plant (harvested seed number), we

divided the total mass of seeds per plant by the estimated seed mass of each plant. The seed mass of each plant (harvested seed mass) was calculated by weighing a single sample of 100 seeds collected from each individual (determined to the nearest microgram).

To avoid confusion we define the following terms: *published seed mass* (Table S1 in appendix) refers to the mean seed mass recorded in the experiments of Alonso-Blanco et al. (1998; 1999); *sown seed mass* (Table S1 in appendix) refers to the mean seed mass of lines obtained from The Arabidopsis Information Resource (TAIR) and weighed before sowing; *total mass of seeds*; *harvested seed mass* and *harvested seed number* refer to data collected for each individual in the experiment described here.

Statistical analysis

We analyzed harvested seed mass, harvested seed number and the total mass of seeds in relation to sown seed mass. Notice, that we use sown seed mass rather than harvested seed mass as the explanatory variable. This is because we wish to assess the fitness consequences of starting life from a particular seed mass. Sown seed mass is also a true fixed variable as required for regression analysis. In many studies, variation in sown seed mass has a large environmental component and such analysis is therefore not possible (Aarssen & Burton 1990; Wulff 1986). All analyses were carried out using linear mixed-effects models in the statistical package R using the *lmer* function (R Development Core Team 2008) which does not perform F-tests, and so we followed the model-building approach outlined in Pinheiro & Bates (2000). For the fixed effects we first assessed the approximate significance of terms using F-tests from a linear model with the appropriate error terms (Table 3). The final significance was assessed using t-tests from the table of coefficients in a mixed-effects model which only retained significant terms. The significance of the random effects was judged using likelihood ratio tests and non-significant terms were removed. The variables harvested seed mass, harvested seed number, total mass of seeds and sown seed mass were all log-transformed to meet the

assumptions of the analysis and because expected relationships are on log-log axes (see Introduction). However, means and differences between means are presented on the original scale. Differences between means are presented with their 95 % confidence interval (CI).

Table 1. Correlations between the mean seed mass recorded for each line in a previously published study (Alonso-Blanco et al., 1999), seeds obtained from the Arabidopsis centre TAIR (sown seed mass) and the experiment reported here (harvested seed mass in two pot sizes). All values are highly significant (P <0.0001, n = 30).

Variables	Published seed mass	Sown seed mass	Harvested seed mass (10 mm diameter pots)	Harvested seed mass (40 mm diameter pots)
Published seed mass	1	0.885	0.850	0.770
Sown seed mass		1	0.778	0.756
Harvested seed mass (10 mm diameter pots)			1	0.870
Harvested seed mass (40 mm diameter pots)				1

RESULTS

Overview

As recommended by Gelman & Hill (2006) we begin by fitting a model for both harvested seed size and harvested seed number with all terms, including pot size, fitted as random effects. This provides a general overview of how the variance is partitioned between the various possible terms and their interactions. The phenotypic variance of a character such as seed mass can be divided into its genetic and environmental components and their interactions (Ridley 2004). In this case, lines represent the genetic component (individuals belonging to the same line are genetically identical) and pot diameter and block represent the environmental components. We express all variance components as percentages.

As expected, most of the variance (67 %) in harvested seed mass is due to lines: i.e. seed mass is under strong genetic control (Figure 2A) which explains the highly significant correlations obtained between our data and previous datasets (Table 1). In contrast, most of the variance in harvested seed number (85 %) is due to pot diameter, i.e. to the environment (Figure 2B). Interestingly, the correlation between seed number in our experiment and a previous dataset are weaker (Table 2), indicating that lines that performed well in our

experiment did not necessarily perform well in a previous experiment. The interaction between the genetic and environmental component appears to be very small in both cases (<1 %, Figure 2).

Table 2. Correlations between the mean seed number recorded for each line in a previously published study (Alonso-Blanco et al., 1999) and the experiment reported here (harvested seed number in two pot sizes). ** Significant values (P <0.05, n = 30), ^{NS} non significant value.

Variables	Published seed number	Harvested seed number (10 mm diameter pots)	Harvested seed number (40 mm diameter pots)
Published seed number	1	0.133 ^{NS}	0.491 **
Harvested seed number (10 mm diameter pots)		1	0.396 **
Harvested seed number (40 mm diameter pots)			1

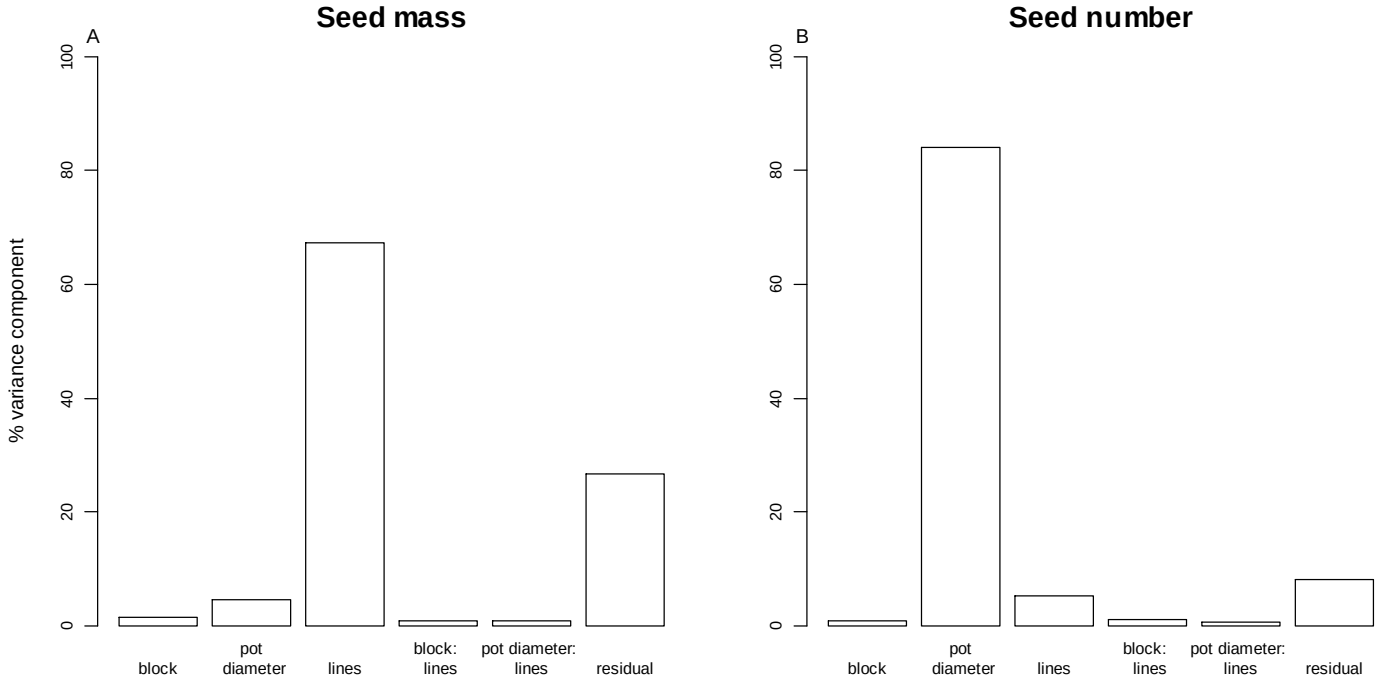

Figure 2. Results of a variance components analysis of harvested seed mass (A) and seed number (B). Variance components are expressed as percentages of the total in each case. Note that seed mass shows a large genetic component (variation among lines) whereas seed number shows a large environmental component (variation between pot sizes) of variation.

Detailed analyses

The relationship between sown seed mass and the total mass of seeds produced (a surrogate for final size) is shown in Figures 3A-B. The total mass of seeds produced in 40-mm diameter pots was 14.4 (CI: 11.7-17.9) times larger than the total mass of seeds produced in 10-mm diameter pots. This is what we expected, as the 40-mm diameter pot had a soil volume exactly 16-times greater than the 10-mm diameter pot. The total mass of seeds produced was unaffected by sown seed mass, so that seed mass and adult mass are entirely uncoupled ($F_{1,29}$ = 1.55, p = 0.223, Table 3A). The total mass of seeds was also not affected by the *erecta* mutation ($F_{1,29}$ = 1.60, p = 0.216, Table 3A). The pot size × lines interaction was effectively zero, but variation among lines was large (χ^2 = 15.3).

The relationship between sown seed mass and harvested seed mass was strongly positive (Figures 3C-D) for both pot sizes. There was an interaction between the *erecta* mutation and pot size ($F_{1,28}$ = 5.72, p = 0.0237, Table 3B). Non-*erecta* lines produced seeds that were on average 0.155 mg (CI: 0.0836-0.231 mg) bigger in 40-mm than in 10-mm pots; a difference of around 16%. In contrast *erecta* lines produced seeds that were on average only 0.0517 mg (CI: -0.00909-0.116 mg) bigger in 40-mm than in 10-mm pots; a difference of around 3.3%. Thus, lines carrying the *erecta* mutation appear to have less phenotypic plasticity in seed size. The slope of the relationship between sown seed mass and harvested seed mass is the same in both pot sizes (0.81 ± 0.217). For the random effects, the pot size × lines interaction was effectively zero, but variation among lines was again large (χ^2 = 65.9).

Given that there was no relationship between seed size and adult size and seed size was strongly conserved, we expected a seed size/number trade-off among individuals to emerge. In fact, the slope of the relationship between sown seed mass and harvested seed number (Figures 3E-F) was very close to the expected value of -1 (see eqn 2; slope: -1.02 ± 0.775). Individuals produced 13.3 (CI: 10.7-16.1) times more seeds in 40 mm pots than in 10 mm pots. Carrying the *erecta* mutation did not affect the number of seeds a plant produced ($F_{1,29}$ = 1.24, p =

0.0275, Table 3C). The pot size × lines interaction was effectively zero, but again variation among lines was large ($\chi^2 = 35.5$).

Table 3. ANOVA of the total mass of seeds (A), harvested seed mass (B) and harvested seed number (C). The appropriate error term is given in each case.

(A)

Term	Error term	Df	Sum Squares	Mean Square	F	P
Pot diameter	R	1	376	376	681	<0.0001
erecta mutation	L	1	2.56	2.56	1.60	0.216
log (Sown seed mass)	L	1	2.48	2.48	1.55	0.223
Lines (L)	R	29	46.3	1.60	2.89	<0.0001
Pot diameter : *erecta* mutation	P:L	1	0.100	0.100	0.167	0.686
Pot diameter : log (Sown seed mass)	P:L	1	0.640	0.640	1.07	0.310
Pot diameter : Lines (P:L)	R	28	16.8	0.600	1.09	0.359
Residual (R)	-	154	85.1	0.550	-	-

(B)

Term	Error term	Df	Sum Squares	Mean Square	F	P
Pot diameter	R	1	0.305	0.305	12.2	<0.0001
erecta mutation	L	1	0.00210	0.00210	0.0125	0.912
log (Sown seed mass)	L	1	11.1	11.1	66.3	<0.0001
Lines (L)	R	29	4.87	0.168	6.73	<0.0001
Pot diameter : *erecta* mutation	P:L	1	0.124	0.124	5.72	0.0237
Pot diameter : log (Sown seed mass)	P:L	1	0.00580	0.00580	0.268	0.608
Pot diameter : Lines (P:L)	R	28	0.605	0.0216	0.865	0.663
Residual (R)	-	154	3.85	0.0250	-	-

(C)

Term	Error term	Df	Sum Squares	Mean Square	F	P
Pot diameter	R	1	354.94	354.94	731	<0.0001
erecta mutation	L	1	2.71	2.71	1.24	0.275
log (Sown seed mass)	L	1	24.1	24.1	11.0	0.00244
Lines (L)	R	29	63.5	2.19	4.51	<0.0001
Pot diameter : *erecta* mutation	P:L	1	0.440	0.440	0.759	0.391
Pot diameter : log (Sown seed mass)	P:L	1	0.520	0.520	0.897	0.352
Pot diameter : Lines (P:L)	R	28	16.4	0.580	1.20	0.237
Residual (R)	-	154	74.8	0.490	-	-

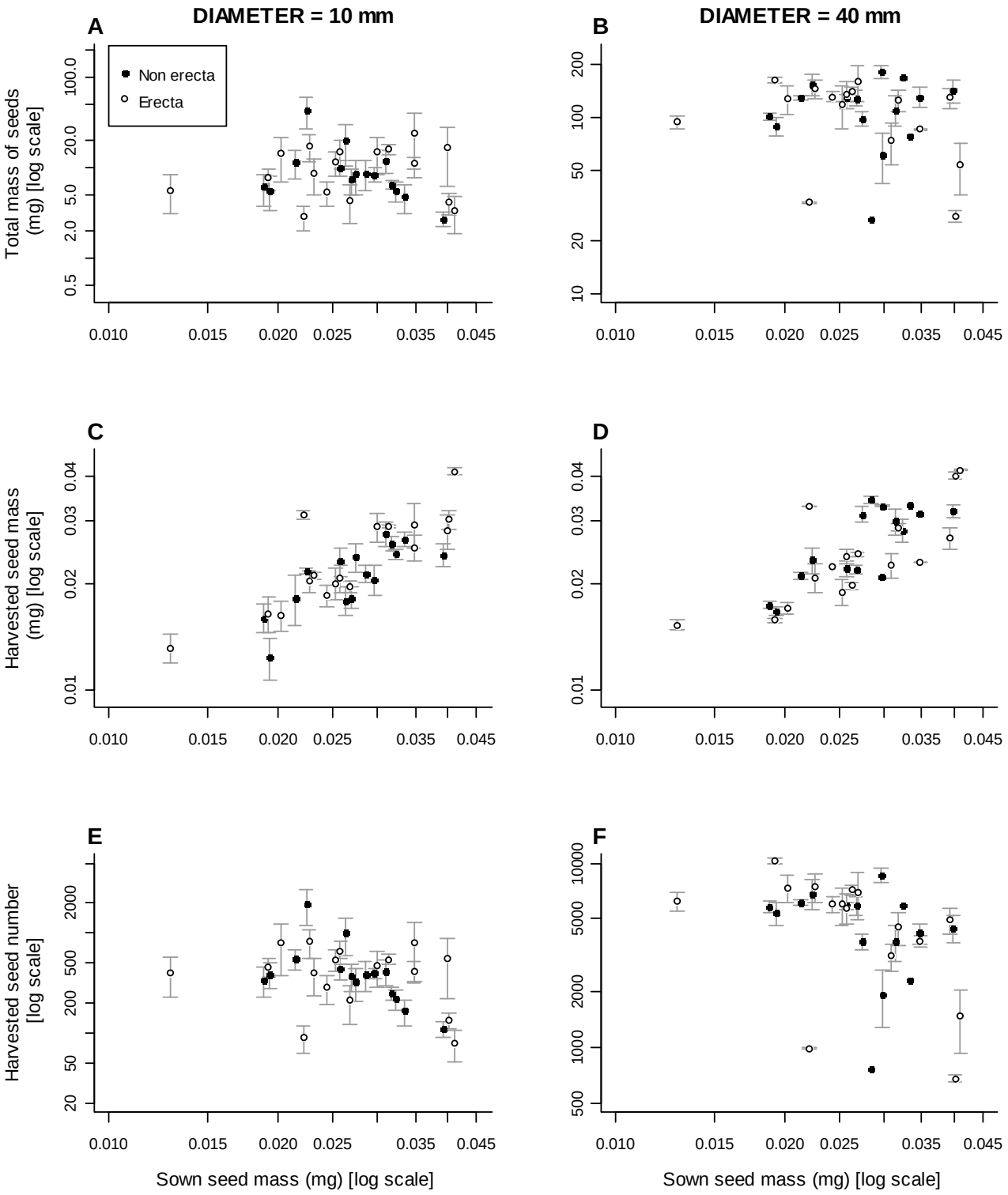

Figure 3. Relationships between total mass of seeds and sown seed mass (A-B), harvested seed mass and sown seed mass (C-D), and harvested seed number and sown seed mass (E-F) in both 10 mm diameter pots (LHS) and 40mm diameter pots (RHS).

DISCUSSION

We grew individual *Arabidopsis* plants from a recombinant inbred population in such a way that belowground resources limited growth and provided two levels of resource availability. On average, the final total mass of seeds was a simple multiple of the available resources, regardless of the initial seed size. Thus seed size did not affect a plant's ability to exploit the available belowground resources. Because there was no relationship between seed size and adult size we found a "perfect" trade-off between sown seed mass and harvested seed number (a slope of -1 on log-log axes). Lines produced slightly larger seeds in larger pots; but this phenotypic plasticity was less pronounced in lines carrying the *erecta* mutation. Venable (1992) proposed that such a plastic increase in seed size is adaptive when there are density-dependent interactions among siblings, as is likely to be the case for *Arabidopsis* which tends to have rather limited dispersal (Wender et al. 2005). However, plasticity in seed number was much larger than plasticity in seed size, as has been observed for many species (Harper et al. 1970; Weiner et al. 1997). Lines differed substantially in the total mass of seeds produced, although such variation was uncoupled with seed size. This is despite the fact that negative correlations between seed mass and some vegetative traits (total leaf number and length of largest leaf) are reported in the original manuscript describing this RIL population (Alonso-Blanco et al. 1999). Such variation in the total mass of seeds produced is perhaps more likely to reflect other differences in this recombinant inbred population such as the timing of flowering (Alonso-Blanco et al. 1998;1999), insect resistance (Kliebenstein et al. 2002) or freezing tolerance (Alonso-Blanco et al. 2005) – which can all have negative effects on growth and may also affect final size.

In our experiments there was no correlation between seed size and adult size. However, such a relationship probably emerges because growth is so strongly constrained by the pot. Thus, small-seeded genotypes can eventually catch up with large-seeded genotypes because

large-seeded genotypes eventually run out of resources and have to stop growing. For example, Susko and Cavers (2008) found that individuals from large seeds were larger after 15 days of growth in pots, but that such differences disappeared at later dates presumably because individuals from smaller seeds begin to catch up once the pot begins to limit growth. Similarly, Turnbull et al. (2008a) found that sand-dune annuals grown in pots had an initial phase of exponential growth during which the initial size hierarchy changes little, followed by a linear growth phase in which small-seeded species effectively close the gap on larger-seeded ones.

However, although there is no relationship between seed size and final size for plants grown in pots, the same might not be true for plants grown in the wild. For example, if all individuals are capable of exponential growth throughout their lives then, other things being equal, adult size is entirely determined by initial size and the trade-off disappears among individuals (see *Introduction*). Within this framework seed size would appear to be a neutral trait (but see Turnbull et al. 2008b) as individuals growing from seeds of all sizes ultimately produce the same number of seeds and hence have the same fitness (eqn 5). Although true exponential growth throughout a plant's life seems unlikely, perfectly size-symmetric competition (Weiner 1985) would yield the same result. In perfectly size-symmetric competition, individuals gain resources in direct proportion to their size. Thus, the size-hierarchy does not change throughout the growth period (Weiner 1985, Weiner 1988, Freckleton & Watkinson 2001). In this case, the ratio of final/initial mass is constant for all seed sizes and all plants have equal fitness. If seed size were a neutral trait and thus free to drift, this might explain why similar species in the same environment have such a large variety of seed sizes (Rees 1995, Levine & Rees 2002). It might also explain why seed size/number trade-offs among individuals are sometimes difficult to detect in natural situations (Mitchell-Olds 1996); because the trade-off now appears among populations and not among individuals.

However, if seed size were a neutral trait and free to drift among species it is difficult to understand the lack of plasticity within species (Harper et al. 1970). The coefficient of variation

(CV) of seed size within species is on average only around 25% (Turnbull et al. 2006) implying that there is strong stabilising selection on seed size as simple theory predicts (Smith & Fretwell 1974; Rees & Venable 2007). Currently, few good explanations exist for the variation in seed size found in *Arabidopsis thaliana* (Alonso-Blanco et al. 1999). However, it is interesting to note that the original parent lines come from very different geographical locations, and that the main source of seed size variation in *Arabidopsis* is likely to be among and not within different populations. For example, the small-seeded Landsberg accession (from Northern Europe) may be a product of a more urban environment, where suitable opportunities may often consist of cracks in pavements or gaps between cobble-stones. Here the amount of soil is limited and adult size is therefore determined primarily by the environment. In these circumstances, small-seeded species will tend to have higher fitness because of the seed size/number trade-off. In contrast, the large-seeded Cvi from tropical Africa is perhaps to be found in more stable environments with intense size-asymmetric competition; thus favouring larger seeds. Further study into the ecological conditions from which the wild-type accessions were originally collected may shed further light on this variation.

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SUPPLEMENTARY INFORMATION

Table S1. Information about the 32 lines selected for the study. The two accessions L*er* and Cvi are the parents. The 30 remaining recombinant inbred lines are derived from reciprocal crosses between the two parents.

NASC	RIL Koornneef	Published Seed Mass (*) [mg]	Sown Seed mass (**) [mg]	*erecta* mutation
N8581	L*er*	0.0193	0.0202	1
N8580	Cvi	0.0351	0.0348	0
N22002	CVL3	0.0162	0.0129	1
N22014	CVL15	0.0145	0.0193	0
N22018	CVL19	0.0251	0.0263	1
N22026	CVL27	0.0275	0.0270	1
N22030	CVL31	0.0295	0.0334	0
N22033	CVL34	0.0236	0.0297	0
N22036	CVL37	0.0325	0.0399	0
N22037	CVL38	0.0150	0.0188	0
N22038	CVL39	0.0202	0.0258	0
N22043	CVL44	0.0242	0.0285	0
N22051	CVL53	0.0327	0.0310	1
N22057	CVL60	0.0286	0.0393	1
N22059	CVL62	0.0190	0.0224	0
N22094	CVL124	0.0274	0.0252	1
N22095	CVL125	0.0200	0.0214	0
N22098	CVL128	0.0273	0.0274	0
N22099	CVL129	0.0243	0.0268	0
N22105	CVL135	0.0327	0.0348	1
N22107	CVL137	0.0302	0.0314	0
N22109	CVL139	0.0217	0.0231	0
N22112	CVL142	0.0315	0.0318	1
N22124	CVL154	0.0317	0.0323	0
N22128	CVL158	0.0373	0.0411	1
N22130	CVL160	0.0361	0.0402	1
N22132	CVL162	0.0256	0.0221	1
N22138	CVL168	0.0334	0.0299	0
N22148	CVL178	0.0207	0.0226	1
N22149	CVL179	0.0223	0.0243	1
N22156	CVL187	0.0183	0.0192	1
N22160	CVL191	0.0280	0.0257	1

(*) Source : Alonso-Blanco et al., 1999.

(**) Source: The Arabidopsis Information Resource (TAIR).

CHAPTER 3

Flowering decisions and environmental sensitivity in *Arabidopsis thaliana*

Cloé Paul-Victor and Lindsay A. Turnbull.

ABSTRACT

An important element in the reproductive strategy for annual plants is the correct timing of the flowering transition. Here we focus on the bolting decision for annuals (the decision to switch from the vegetative to the reproductive phase) and consider whether plants make the switch at an optimal time. We consider two main growth and reproduction models based on logistic growth for annual plants diverging mainly in reproductive mass allocation and in the length of vegetative activity. We used natural variation in *Arabidopsis thaliana* and performed a whole-plant partitioning study, using sequential harvests, to characterize growth, while also recording the timing of flowering. We used 30 RILs from a commonly studied RIL population (Cvi x L*er*) and one of its parents, the wild type line Landsberg *erecta* (L*er*). In an experiment using three different pot sizes to provide different degrees of belowground growth restriction, we show that bolting later does not allow the plant to achieve a higher total mass or accumulate more resources. Leaf production ceases when bolting is initiated, indicating a hard switch from vegetative to reproductive growth. Our results show that on average only 50 % of the total mass accumulated by a plant occurs before bolting is initiated and rosette growth stops. Although there is no further investment in the rosette, the rosette is still physiologically active. We also demonstrate that on average plants initiate bolting at the inflection point of the logistic growth curve when their absolute growth rate reaches a maximum and thus further investment in vegetative parts would not lead to further increases in growth. The *Arabidopsis* plants thus seem to possess the ability to sense the environment by initiating bolting when their intrinsic growth cannot be maximized anymore. It seems that delaying flowering does not lead to higher reproductive mass when a plant has a fixed amount of resources available in the environment. We did not observe a rigid “clock”-like strategy but rather an optimal solution involving sensing the environmental cues.

Background review about *Arabidopsis thaliana* flowering

A major developmental transition in annual flowering plants is the switch from vegetative to reproductive development. The correct timing of this transition is essential to maximize reproductive success (Simpson and Dean 2002). The flowering decision is even more crucial for annual plants whose offspring production relies on this switch under favourable conditions. Physiological and genetic analysis of flowering has shown that multiple environmental and endogenous inputs influence the timing of the switch (Boss et al. 2004). Flowering at the right time requires the perception and the processing of a diverse range of environmental and internal signals (Putterill et al. 2004) requiring plants to specialize in sensing environmental stimuli and adapting their development accordingly (Werner et al. 2005). Plant species exhibit great variability in flowering time, they have to align their life history with favourable environmental conditions (Henderson and Dean 2004) as well as respond to changes in their local environment (competitions for nutrients, light, Putterill et al. 2004). Our knowledge of the genetic regulation of flowering time in the model plant *Arabidopsis thaliana* has rapidly increased recently (Roux et al. 2006). Flowering time is a quantitative trait controlled by multiple genes (Roux et al. 2006) and there have been numerous and recent reviews on various aspects of flowering control (Simpson and Dean 2002, Boss et al. 2004, Putterill et al. 2004, Werner et al. 2005, Roux et al. 2006). Five main flowering-time pathways had been identified, either promoting or enabling the floral transition (Boss et al. 2004) :

- The photoperiod pathway: utilizing photoreceptors in conjunction with the circadian clock to strongly accelerate flowering in the presence of long day photoperiods (Werner et al. 2005). This is a pathway that promotes floral transition (Boss et al. 2004).

- The light quality pathway: light also affects flowering time independently of photoperiod via light quality pathway (Simpson and Dean 2002, Boss et al. 2004). Light quality is affected by shading, which results in reduction in the ratio of red to far-red light (Boss et al. 2004). Overcrowding is also detected by changes in light quality (Putterill et al. 2004).
- The vernalization pathway : in order to prevent precocious flowering when conditions for reproductive development may not be favourable (Putterill et al. 2004), the plants need to be exposed to an extended period of winter-like temperatures, a process called vernalization (Werner et al. 2005). This pathway enables the flower transition (Boss et al. 2004). Putterill et al. (2004) suggested epigenetic changes in chromatin structure to be at the basis of the cellular memory of vernalization to explain how plants that have been vernalised remember this signal and flower maybe months later.
- The hormones pathway: hormones of the gibberellin class promote flowering in the absence of positive cues from the photoperiod pathway (Werner et al. 2005). This pathway promotes flowering (Boss et al. 2004).
- The autonomous pathway: is the internal signals regulating the plant (Putterill et al. 2004), which was originally thought to function independently of the environment. Recently, however, it has been found that this pathway may also mediate response to ambient growth temperature (Blazquez et al. 2003, Werner et al. 2005).

In addition to the pathways described above, there is an increasing list of genes that have been classified as floral repressors. During early vegetative development, floral repressors in the enabling pathways overcome any promotive cues, ensuring that a sufficiently long vegetative phase occurs for the necessary energy to be accumulated. During the later stages of vegetative development, the activity of the floral repressors declines and there is a progressive activation of floral promoters until a quantitative threshold is reached and the transition of the meristem from a vegetative to a reproductive state occurs. The pattern

of gene expression in the flowers then must be reset in the gametes and developing embryos so that the next generation can determine its own « right » time to flower (Boss et al. 2004).

The interaction of these different pathways changes in response to different environmental and endogenous cues to generate the plasticity and diversity of the flowering response (Boss et al. 2004). The trade-off between resource accumulation and risk avoidance for offspring production (producing early enough before unfavourable conditions) is the main challenge for the plants. In the following chapter we consider how the decision to flower can be optimised.

INTRODUCTION

Maximizing the yield of seeds is crucial for annual plants. One important element in the reproductive strategy is the correct timing of the flowering transition (Simpson and Dean 2002). To maximize successful total seed production, plants must be able to flower under favourable environmental conditions, and the proper timing of flowering therefore has an important adaptative value for plants (Koornneef et al. 1998, Obeso 2002). Thus, central questions include: 1) when would be the optimal time to flower given the environmental constraints and 2) how do plants respond to variation in the availability of resources in their environment? These central questions have been addressed in numerous studies but the optimality of the onset of flowering is largely unknown (King and Roughgarden 1983).

The transition from vegetative to reproductive development is controlled by both environmental and endogenous factors (Koornneef et al. 1998, Putterill et al. 2004). Various models have been developed to study growth and reproduction in annual plants with the partitioning of resources between vegetative and reproductive functions. Such models have considered the consequences of environmental stochasticity (King and Roughgarden 1982); the effect of competition between individuals (Gadgil and Gadgil 1975, Schaffer 1977, Mirmirani and Oster 1978), the effects of seasonal variations in environmental quality (Paltridge and Denholm 1974, Denholm 1975, King and Roughgarden 1982) and the effect of nutrient level or water availability (Pigliucci et al. 1995, McConnaughay and Coleman 1999). More recently, genetics has been used to study the mechanism of flower initiation (*Background review*). Despite all theses studies, theoretical papers rarely relate predicted strategies to measured values of reproductive effort in the literature (King and Roughgarden 1983). Another questionable point is a lack of information about direct estimates of plant fitness, which is rarely assessed as life-time production of viable seeds (most of the plant fitness estimations measured some life-history parameters related to fitness, Obeso 2002).

Therefore the growth models are mostly based on vegetative biomass or organs biomass and not on the total seed production.

Our main goal is to determine the optimal time for flowering initiation given environmental constraints in *Arabidopsis thaliana*. We will focus on two main models for growth and reproduction specific to annual plants in absence of competition. We call the first model the *traditional flowering model* and the second model the *dynamic optimisation model*. Assumptions of both models are explained in details below.

Vegetative versus reproductive allocation

Theory

A model of growth and reproduction in annual plants was first developed by Cohen (1971) to determine the allocation strategy which maximizes seed yield. The model divides the plant into vegetative and reproductive parts and predicts that yield is maximized by a strategy consisting of a switch from purely vegetative to strictly reproductive growth. This model did not include how the initiation of flowering was triggered, whether the vegetative part continued to be active after the transition and how the date (plant age) of transition affected the seed production (fitness) of the plants.

According to Cohen (1976), in general, the growth of the biomass of most plants can be described by a logistic curve (Figure 1a). The absolute growth rate continuously increases until the inflection point of the curve is reached. At this point, the growth rate starts to decrease until the asymptotic mass is reached. These two parts of the plant growth curve (corresponding respectively to increasing and decreasing absolute growth rates) can be more easily seen by taking the derivative of the logistic function (Figure 1b).

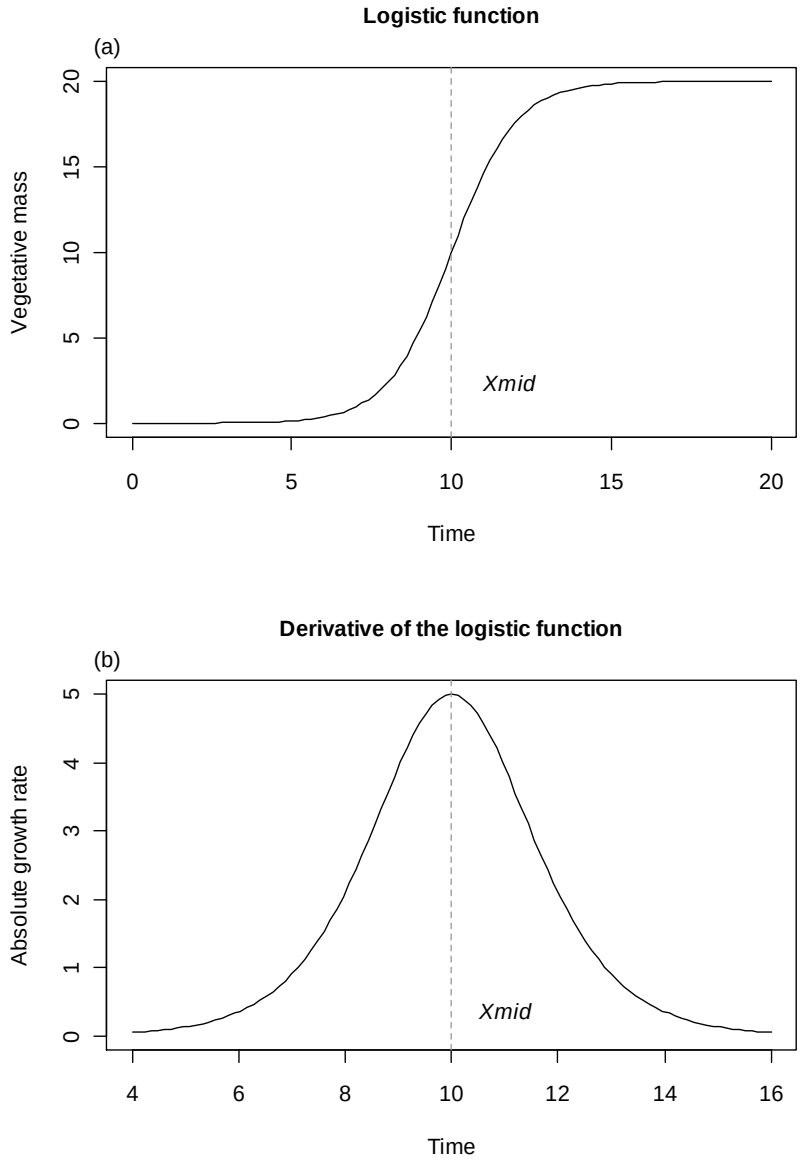

Figure 1. Graphics representing (a) total growth (logistic function) and (b) the absolute growth rate (derivative of the logistic function). The dotted vertical lines of both graphics represent the inflection point of the logistic curve (*Xmid*) which corresponds to the maximal absolute rate of the plant. The logistic curve (a) is commonly used as a good representation of total growth. Its derivative (b) shows how absolute growth rates change during the growth curve. The inflection point (*Xmid*) defines the point at which the absolute growth rate is maximal.

The reason that growth rates decline is presumably due to decreasing efficiency per unit biomass in gathering resources and by a continuously increasing cost of maintenance per unit biomass (Cohen 1976). This logistic growth was also used by Hunt (1982) to describe plants grown in pots. In the simplest case, the expected mass (M) of an isolated individual in the absence of competition is:

$$M = \frac{KS\exp(\alpha t)}{K + S(\exp(\alpha t) - 1)} \quad \text{(eqn 1)}$$

where K is the maximum adult size that a plant can attain, S is the seed size (i.e. the initial plant size) and α is the intrinsic growth rate. This logistic model for growth is the basis of the two following models although they diverge in the carbon source for the reproductive parts and in the length of vegetative activity.

The traditional flowering model

According to Mitchell-Olds (1992) larger size is often associated with greater fecundity in annual plants. A study on *Arabidopsis thaliana* showed that flowering time is positively correlated with size at first reproduction measured by leaf number (Mitchell-Olds 1996). Later, a study about *Arabidopsis thaliana* insect resistance (Kroymann et al. 2003) where the growth rate was quantified, stated that "Juvenile biomass is positively correlated with individual fitness in *Arabidopsis…"* referring to the study of Mitchell-Olds (1996). Here fitness is used as the fecundity per individual or total fruit number per individual as Mitchell-Olds defined it in his study (1996). However, the positive correlation between biomass at flowering and total seed number per individual was not directly demonstrated. This positive correlation was clearly demonstrated only between flowering time and number of leaves. This assumed positive correlation between vegetative biomass (number of leaves) and fecundity (seed production) means that the later a plant initiates flowering, the greater will be its fecundity. An early-flowering plant would have less resource accumulated to shunt into its reproductive part. In contrast, a late-flowering plant would have accumulated more resource, hence a greater seed production, because had simply more time. It implies 1) that the vegetative part stops any activity or matter accumulation when the switch to reproductive phase (or flowering initiation) occurs, 2) that all resources to build up the reproductive part come from the sole remobilization of the vegetative part. According to this model, two plants

with different flowering time (an early flowering plant and a late flowering plant) would result in two distinct reproductive biomasses.

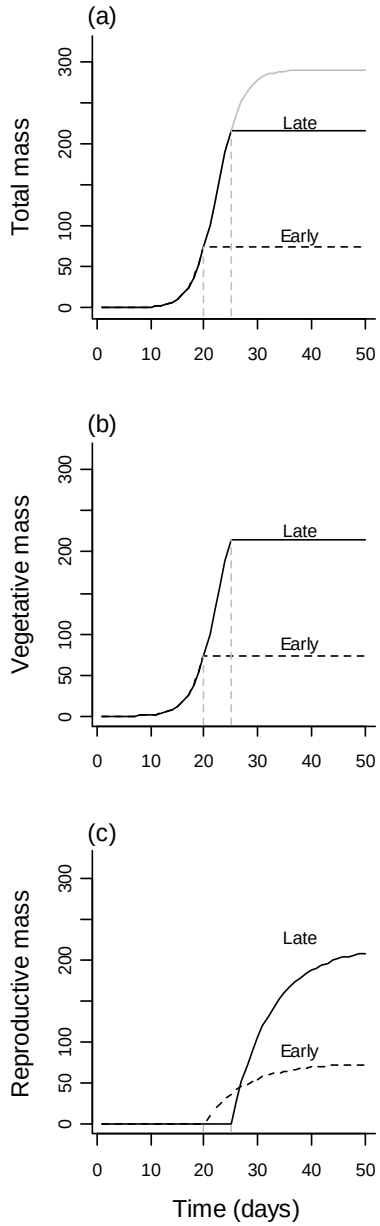

Figure 2. The *traditional flowering model* showing late-flowering (Late) and early-flowering (Early) plants. (a) The potential total mass that a plant can accumulate from a pot assuming no additional nutrient input is shown by the solid grey line. Once flowering is initiated, all growth is assumed to stop. Thus, early flowering results in lower total mass accumulation. (b) The vegetative growth therefore follows the same pattern as for total mass (the decline in vegetative mass following flowering is not shown). (c) The reproductive mass therefore begins to accumulate once flowering begins and consists entirely of mass shunted from the vegetative parts. The conversion efficiency is here assumed to be 100%. Late-flowering lines have an enormous advantage in terms of seed production over early-flowering lines.

To see this more clearly, we first assume that plant follows a logistic growth curve (Figure 1a). As plant growth is assumed to stop once flowering is initiated, an early flowering plant is expected to achieve a lower total mass than a late flowering plant (Figure 2a), because once flowering is initiated all growth stops. The late flowering plant has more time to accumulate resources and therefore follows the growth curve longer and has a higher total final mass. The total biomass of these two different plants is the biomass achieved at flowering initiation and thus the total plant biomass at flowering initiation is exactly equal to the vegetative biomass (Figure 2b). At this stage, the reproductive part starts to grow and is derived solely from matter shunted from the vegetative part. If remobilization from the vegetative part to the reproductive part is 100 % efficient, then we expect to have a reproductive part the same size as the vegetative part. An early flowering plant, which accumulated a smaller amount of vegetative biomass than a late flowering plant, would in consequence produce a smaller reproductive part (Figure 2c). Here, late-flowering plants present an incontestable advantage over earlier-flowering plants. Under this model, late-flowering plants would always have to higher reproductive output.

The dynamic optimisation flowering model

New techniques in engineering were developed allowing calculation of optimal design (Iwasa 2000). Formal calculations of dynamic optimisation were then introduced into flowering models and such models were termed dynamic optimisation models (Taylor et al. 1974, Leon 1976, King and Roughgarden 1982, 1983). For example King and Roughgarden (1982) generalized Cohen's model to include vegetative and reproductive loss terms. Finally, Iwasa (2000) presented a review of how the growth and reproductive schedule of plants can be usefully studied as the dynamic optimal allocation of material between different organs.

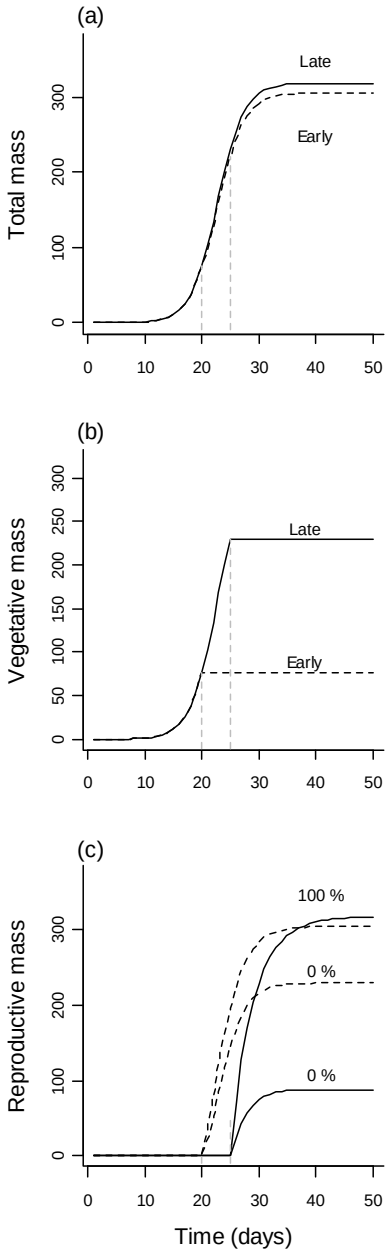

Figure 3. The *dynamic optimisation model* showing late-flowering (Late) and early-flowering (Early) plants. (a) Carbon fixation continues after flowering begins, although no further carbon is allocated to vegetative structures. Flowering any time after the inflection point should not change the future accumulation of total mass. However, flowering before the inflection point may decrease future growth rates and potentially the asymptotic mass (Early). (b) The accumulation of vegetative mass shows the same pattern as for the standard model, with vegetative allocation ceasing as soon as flowering is initiated. (c) Reproductive mass then depends on the conversion efficiency of vegetative mass into reproductive mass. When this is zero (0 %), the reproductive structures consist only of carbon fixed after flowering begins; hence early-flowering lines actually outperform late-flowering ones. However, when this conversion efficiency is 100%, later-flowering lines have a small advantage over early-flowering lines, the size of which depends on the extent to which early flowering compromises future growth rates.

According to Iwasa (2000), in his description of the *dynamic optimisation model* for annual plants, the vegetative part contributes to the reproductive success by photosynthesis continuing until the end of the season. Physiological activity of the vegetative part does not stop when flowering is initiated, despite a hard switch from vegetative to reproductive allocation. Thus, the main difference with the "traditional flowering model" is the continuation of activity in the vegetative part despite the cessation of allocation. Although no further growth occurs in the vegetative parts, this activity continues to contribute to the reproductive growth. In this case, both early-flowering plants and late-flowering plants continue to follow the logistic growth curve and total mass continues to accumulate after flowering initiation (Figure 3a). If plants flower anytime after the inflection point of the growth curve (i.e. the point where the absolute growth rate is maximum Figure 1) no change should be observed for the future accumulation of total mass. However, if the plants flower before the inflection point (i.e. when the absolute growth rate is below maximum) then it may decrease its future growth rate and potentially the final total biomass, for example it may reach a lower mass.

As the vegetative biomass stops accumulating at flowering initiation, we observe the same pattern of vegetative mass accumulation as in the *traditional flowering model* (Figure 3b), i.e. it stops once flowering is initiated. In contrast, the reproductive biomass shows a very distinct pattern from the *traditional flowering model*. In the *traditional flowering model* the remobilization efficiency from the vegetative part to the reproductive part was assumed to be complete (100 %). Here we present two extreme possibilities, one with no remobilization from vegetative to reproductive parts (0 %) and one with complete efficiency (100 %). When remobilization is 100 % efficient (as in the *traditional flowering model*) the late-flowering ones might only have a slight advantage over the early-flowering ones (assuming early-flowering has little effect on future growth). The advantage late-flowering plants depends on the growth rate decrease of the early-flowering plants. According to how early they flower,

the penalty can be more or less pronounced (Figure 3c). When the conversion efficiency is 0 %, then the reproductive part consists solely of new carbon fixed by rosette activity after flowering is initiated (Figure 3c). In this case, early-flowering plants may actually have an advantage over late-flowering plants. This occurs because resources locked up in the vegetative part cannot be shunted to the reproductive parts and late-flowering plants lock up more resources by continued vegetative growth.

Consequences of the "dynamic optimisation flowering model" assumptions

According to Iwasa (2000) the switch from vegetative to reproductive part (i.e. flowering initiation) occurs when the investment in vegetative growth is not sufficient anymore to pay back investment costs. In our case, where plants are belowground limited, the plant should continue to invest new resources into vegetative parts as long as it continues to ensure a higher absolute growth rate. When this condition is not met, investment in further leaf production should stop and plant should flower. Flowering earlier than this is likely to be suboptimal as it leads to reduced future growth, hence it would take longer to extract available resources from the environment because of a slower absolute growth rate. Flowering later than this optimal could also have a cost and be suboptimal. If the plant continues to accumulate mass in vegetative structures when is not necessary, it might incur the costs of remobilization when the moment comes to convert leaves into seeds.

Under the *dynamic optimisation flowering model*, if a plant grows logistically then this optimal switch would occurs when the absolute growth rate is maximal i.e. the inflection point of the logistic curve (Figure 1). At this point the plant should have reached only half of its total final biomass. This is rather different from the *traditional flowering model* from Mitchell-Olds (1996) where the total growth stops completely when the plants flowers and vegetative mass is converted into seeds. Under the *traditional flowering model*, later flowering would always lead to higher reproductive output. In the dynamic optimisation

models, early-flowering plants might actually produce more seeds in total, depending on the efficiency of converting vegetative mass into reproductive mass.

Flowering rules

We have seen the two main models for allocation in plant growth. Here we present the different "rules" a plant might follow to initiate the switch from vegetative from reproductive growth given good growth conditions.

Age and size rule

Plants might follow an "age" or a "size" rule, where they flower at a particular age or size, regardless environmental conditions. Traits related to the timing of flowering influence resource allocation and individual fitness (Widen 1991, Sandring et al. 2007, Franks and Weis 2008). Age and size at first reproduction especially, are thought to be primary life-history traits under selection (Lotz 1990). These rules involve strong genetic constraints (Stearns and Koella 1986, Lotz 1990). The flowering age would therefore be independent from the three parameters (K, S and α, see S2 appendix) of the growth function from Hunt (Hunt 1982). The *traditional flowering model* might use an "age" or "size" rule. Stearns et al. (1986) studied age and size at maturity and assumed in their model that fecundity increases with size. They found that most organisms should mature neither at fixed size nor at a fixed age, but along an age-size trajectory. Furthermore, Sandring et al. (2007) found in a study with *Arabidopsis lyrata* that flowering initiation was not correlated with the size of the rosette.

Growth rate rule

Finally plants might follow a "growth rate" rule, where the bolting initiation is environmental sensitive. This rule presents more flexibility than the two others. This is what the "dynamic optimisation model" might use. According to Iwasa (2000) the switch from vegetative to reproductive part occurs when the investment in vegetative growth is not sufficient anymore to pay back investment costs. This happens at the inflection point of the curve (*Xmid*). Then, we expect flowering initiation (i.e. bolting) at *Xmid*. Furthermore, the inflection point of the curve is dependant on the three parameters of the growth function (*K*, *S* and α, see S2 in appendix) as *Xmid* is defined as:

$$Xmid = \frac{1}{\alpha} \ln\left[\frac{K}{S} - 1\right] \qquad \text{(eqn 13 in appendix 2)}$$

In this model, seed mass (*S*) influences the flowering initiation. According to this equation, small-seeded plants take longer to initiate flowering than large-seeded plants. Iwasa (2000) also predicts the switch from vegetative to reproductive growth to occur late in favourable growing conditions. In contrast, the model predicts the plant to switch earlier in a less favourable growth environment. The "growth rate" rule allows plants to use the same rule but they could initiate flowering at different time because of different values of the parameters α, *K* and *S* according to pot sizes or different environmental conditions between plants.

Aim of the study

We focus on bolting decision (the decision to switch from vegetative to reproductive phase) in *Arabidopsis thaliana*. We aim to investigate the following questions:

a) Does flowering initiation coincide with mid-point of the total mass growth curve (i.e. the inflection point of the logistic curve)?

b) Does total mass accumulation cease after flowering initiation? Do resources continue to accumulate post flowering initiation/cessation of rosette growth? Does timing of initiation of flowering coincide with stopping of rosette growth?

c) Does initiating flowering later lead to a higher asymptotic mass as the *traditional flowering model* predicts (Mitchell-Olds 1996)?

d) How sensitive and plastic is the flowering switch for *Arabidopsis thaliana*? Do the plants use a strict "clock" or "age" rule with a very rigid, highly constrained flowering time or do the plants have the ability to "sense" environmental cues and change their flowering time accordingly.

MATERIAL AND METHODS

Plant material

We used natural variation in *Arabidopsis thaliana* to perform a whole-plant partitioning study, using sequential harvests, to characterize growth. We used 30 RILs from a commonly studied RIL population (Cvi x L*er*) (Alonso-Blanco et al. 1998, Alonso-Blanco et al. 1999) and one of its parents, the wild type line Landsberg *erecta* (L*er*). Three pot sizes were used to provide different degrees of belowground growth restriction. We also used the seed mass information and relate growth to seed output. The age at inflorescence emergence (bolting) was chosen as the starting point of the reproductive phase rather than the age at first flower for our experiment.

The plant material used for this experiment was the same as the set of lines used in a previous experiment (Chapter 2). This is a set of 30 RILs derived from reciprocal crosses between the two pure lines Landsberg *erecta* (L*er*), obtained as a mutant (*er*) from an accession of northern Europe (Rédei 1962, 1992), and Cvi, an accession from the tropical Cape Verde Islands (Lobin 1983). We grew 30 RILs plus the two parent lines for the experiment described here. These RILs present the main advantage of revealing phenotypes outside of

the parental range of variation, thus maximising the range of phenotypic expression (Alonso-Blanco and Koornneef 2000). Using data collected by Alonso-Blanco et al. (1999), this population shows a significant negative relationship ($F_{1,159}$ = 66.9 and p > 0.0001) between flowering time and seed mass with a slope of -0.69 (± 0.046). The 32 lines selected for our experiment are shown among the Alonso-Blanco data (Figure 4).

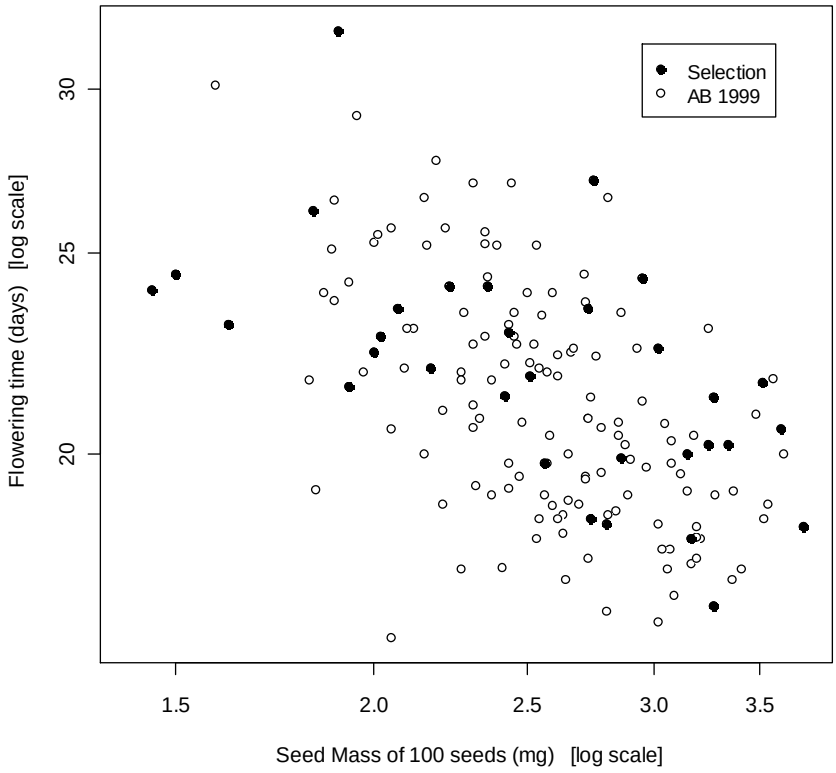

Figure 4. Relationship between the flowering time and the seed mass of the 162 lines from the L*er*-Cvi population, plus the two parents Cvi and L*er* with Alonso-Blanco data (Alonso-Blanco et al., 1999). The complete RIL population is shown with lines we selected for our experiment highlighted (filled circles).

Our lines do not significantly differ from the whole population ($F_{1,157}$ = 2.32 , p = 0.130) with a slope of -0.74 (± 0.075). This negative relationship between flowering time and seed mass illustrates that smaller seeded-lines are sometimes observed to flower later, consistent with the idea that they take longer to extract resources from the pot and hence take longer to reach the inflection point (see *Growth rate rule*). The details about the lines are described fully in

the previous experiment (see Chapter 2). A summary of these lines information is available in Table S1 in the appendix.

Experimental design

The seeds were obtained from The Arabidopsis Information Resource (TAIR) and we weighed one batch of 100 seeds from each of the 32 selected lines. This is referred to as *sown seed mass*. All seeds were then placed in a cold room at 4 °C for one week to synchronise germination. Plants were grown in small (20 mm diameter), medium (30 mm diameter) and large cylinders (40 mm diameter) inserted into standardized cells (65 mm diameter) within a flat completely filled with a mixture of 50% sand and 50% compost. Each flat contained 35 cells and was 70 mm deep. The cylinders allowed us to randomise pot diameter treatments within flats and ensured that the spacing of individuals in different pot sizes and the surface area available to growing rosettes was exactly the same. However, the three pot sizes provide different degrees of belowground growth restriction. At each harvest there were two replicates of each line and pot size combination.

Pots were sown with four seeds and thinned as soon as seedlings emerged to leave one plant per pot (the most central healthy seedling). The plants were grown in a glasshouse with both natural light and additional artificial lighting which came on automatically when the natural light was below 25 kLux and kept under a cycle of 16 h light (22°C) and 8 h dark (20°C). Germination, bolting (initiation of the flowering stem) and flowering (opening of the first flower) were recorded for each plant to the nearest day. On each day plants were checked for a sign of bolting i.e. flowering stem emergence. This day corresponds most closely with the decision by the plants to initiate reproduction. Bolting age was then calculated as:

$$\text{bolting age} = \text{bolting day} - \text{germination day};$$

and the flowering age as:

$$\text{flowering age} = \text{flowering day} - \text{germination day}\,.$$

The dry biomass was collected during six sequential, destructive harvests. We separated the plant parts for weighing into roots, rosette leaves and inflorescence (when there was one). Plants were dried at 80°C for three days and weighed to the nearest microgram. We focussed on the active stages of plant growth (mostly the vegetative phase) by harvesting at relevant points of the plants' development. Each harvest represents a developmental stage observed in most of the plants (Figure 5).

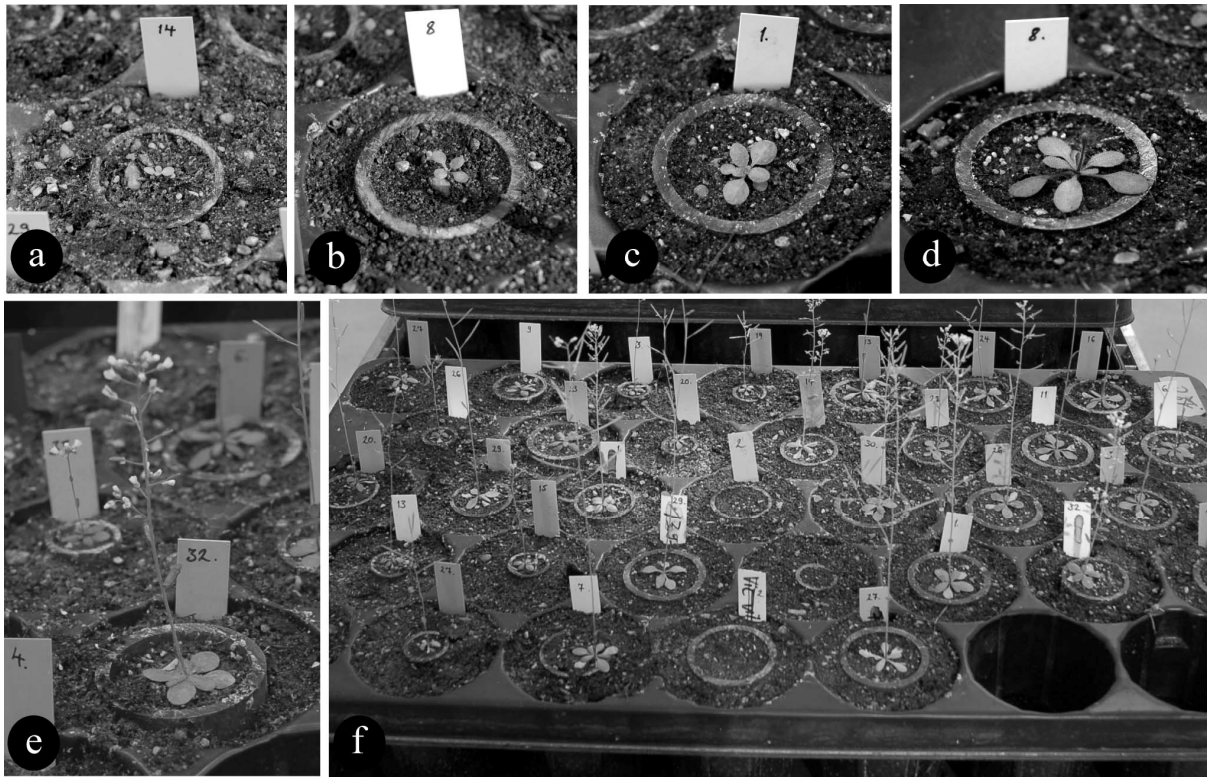

Figure 5. Picture of the experiment showing the developmental stages of the plants at each of the six harvests. 1a. First harvest at 7 days after sowing (DAS) with a two leaves stage. 1b. Second harvest at 11 DAS with a four leaves stage. 1c. Third harvest at 15 DAS with a six leaves stage. 1d. Fourth harvest at 20 DAS when the inflorescence starts bolting with a eight leaves stage. 1e. Fifth harvest at 28 DAS when the flowering starts. 1f. Sixth harvest at 33 DAS when the fruit production starts, showing the 20, 30 and 40 mm diameter cylinders inserted into cells within a single flat. Note that the surface area available to grow rosettes is exactly the same for all treatments.

The first harvest took place 7 days after sowing (DAS) when most plants had only two leaves. The second harvest took place 11 DAS when most plants had four leaves. The third harvest took place 15 DAS when most plants had six leaves. The fourth harvest took place 20

DAS when the plants started to bolt and had on average eight leaves. The fifth harvest took place 28 DAS when the first flowers were seen. The sixth harvest took place 33 DAS when the first fruits appeared. Even at the last harvest no siliques were observed to have opened and hence no biomass was lost as seeds. However, the rosettes were observed to have partially senesced. The number of leaves of each plant for all harvests was also counted.

Statistical analysis

We fitted non-linear mixed-effects models using the function *nlme* in the statistical package R (R Development Core Team 2008). Lines were treated as a random effect and pot volume, seed mass and the *erecta* mutation as fixed effects. Pot volume was log-transformed and fitted as a continuous variable.

Throughout, we followed the model-building approach advocated by the developers of *nlme* (Pinheiro and Bates 2000) which includes assessment and removal of non-significant terms. The significance of fixed effects (pot volume, seed mass and the *erecta* mutation) was assessed using F-tests while the significance of the random effects (lines) was assessed using likelihood ratio tests. For the analysis of total biomass, residual plots showed clearly that the variance increased with the mean. This can be alleviated by using varPower(). Because of its flexibility, the varPower function is a common choice for modelling monotonic heteroscedasticity (Pinheiro and Bates 2000). The heteroscedastic model provided a much better representation of the data.

We modelled total biomass as a function of plant age (days since germination) using a three-parameter logistic model parameterised in the following way:

$$y(x) = \frac{Asym}{1 + \exp\left[\left(Xmid - x\right)/scal\right]} \qquad \text{(eqn 1)}$$

where *Asym* is the horizontal asymptote as $x \to \infty$, *Xmid* is the inflection point of the curve, i.e. the value of x for which $y = Asym/2$, and *scal* is a scale parameter on the x-axis (if *scal*

< 0 the curve will be monotonic decreasing instead of monotonic increasing). Thus, *Asym* is the estimated asymptotic mass of the plant and *Xmid* is the inflection point of the curve, which we expect to coincide with the appearance of the flowering stem. The parameter $scal = 1/\alpha$ where α is the intrinsic growth rate in eqn 14 (see *S2* in appendix). Thus, when the intrinsic growth rate α is high, *scal* is small and vice versa. The correspondence between the more common formulation of the logistic growth curve in the introduction and in Hunt (1982) and the one from Pinheiro and Bates (2000) is explained in appendix S2.

We modelled the number of leaves as a function of plant age using a three-parameter asymptotic function:

$$y(x) = Asym + (R0 - Asym)\exp[-\exp(lrc)x] \qquad \text{(eqn 2)}$$

where *Asym* is the horizontal asymptote as $x \to \infty$ i.e. the asymptotic number of leaves produced, *R*0 is the response at *x* = 0 i.e. the initial number of leaves, and *lrc* is the logarithm of the rate constant corresponding to $t_{0.5} = \log 2/\exp(lrc)$, where $t_{0.5}$ is the half-life i.e. the time after which half the asymptotic number of leaves are produced. Both these functions have self-starting routines (SSlogis and SSasym) and have been designed for easy use (Pinheiro and Bates 2000). After fitting these models, we can predict the total mass or the number of leaves at any time of the development of the plants during the period of our experiment. For example we can estimate the number of leaves or the total mass of the plants when flowering is initiated. We used the age of the plant rather than the time from sowing as we had recorded the germination day for each individual. All estimates are given with 95 % confidence intervals and are taken from the final model in each case.

RESULTS

Calculating the model parameters and fitting models

Total mass

The presence of the *erecta* mutation never had a significant effect on any of the model parameters and hence was removed from the final model (*Asym*: $F_{1,1034} = 0.00008$, $p = 0.993$; *Xmid*: $F_{1,1034} = 0.027$, $p = 0.87$ and *scal*: $F_{1,1034} = 0.232$, $p = 0.631$). The growth of the plants was therefore not affected by carrying the *erecta* mutation. None of the parameters were significantly related to seed size (*Asym*: $F_{1,1034} = 2.282$, $p = 0.1312$; *Xmid*: $F_{1,1034} = 2.653$, $p = 0.1036$ and *scal*: $F_{1,1034} = 0.536$, $p = 0.464$), so seed size was removed from the final model as well.

In contrast, pot volume had a significant effect on all the parameters (*Asym*: $F_{1,1034} = 6.74$, $p = 0.0096$; *Xmid*: $F_{1,1034} = 56.5$, $p < 0.0001$ and *scal*: $F_{1,1034} = 4.60$, $p = 0.0323$). The estimated asymptotic mass (*Asym*) increased with pot volume with an average of 8.58 mg (CI 95%: 7.52 – 9.64) for the 20 mm pots and 32.2 mg (CI 95%: 31.2 – 33.3) for the 40 mm pots (Table 1). Thus lines grown in bigger pots produced greater final total mass. The time to achieve half this mass (*Xmid*) also increased with pot volume. However, the difference between our smallest and largest pots is relatively small: 13.7 (CI 95%: 13.1 – 14.2) days on average for the 20 mm pots and 16.8 (CI 95%: 16.3 – 17.3) days for the 40 mm pots (Table 1), a difference of 2.9 days. The parameter *scal*, whose inverse determines the rate at which the asymptote is approached, decreased with pot size, i.e. the growth rate increased with pot size with an average of 0.314 $mg.day.mg^{-1}$ (CI 95%: 0.299 – 0.331) for the 20 mm pots and 0.339 $mg.day.mg^{-1}$ (CI 95%: 0.322 – 0.359) for the 40 mm pots (Table 1). Thus the plants grew faster in bigger pots.

There was a large effect of line on both *Asym* and *Xmid* (*Asym*: $\chi^2 = 8.862$, $p = 0.0029$ and *Xmid*: $\chi^2 = 46.75$, $p < 0.0001$). So there was variation among the lines for the asymptotic mass (*Asym*) and the time to reach half this mass (*Xmid*). But there was no effect of line identity on the parameter which determines growth rate at a given size (*scal*: $\chi^2 = 1.87*10^{-5}$, $p = 0.997$). It was therefore removed from the final model. The table of estimates for the final

logistic model of total growth is shown in Table 1 and the data plotted together with the model fits in Figure 6.

Table 1. Estimates of fixed effects from the final logistic model (non linear mixed-effects model) fitted to the total mass against plant age. Non-significant terms involving *erecta* mutation and seed mass were removed. Random, i.e. line effects were retained for both *Asym* and *Xmid.*

	Value	Std.Error	DF	t-value	p-value
Asym.(Intercept)	-89.6	3.16	1037	-28.4	<.0001
Asym.log(pot.vol)	17.1	0.529	1037	32.2	<.0001
xmid.(Intercept)	0.752	1.75	1037	0.429	0.668
xmid.log(pot.vol)	2.25	0.263	1037	8.54	<.0001
scal.(Intercept)	4.16	0.540	1037	7.71	<.0001
scal.log(pot.vol)	-0.171	0.0802	1037	-2.13	0.0335

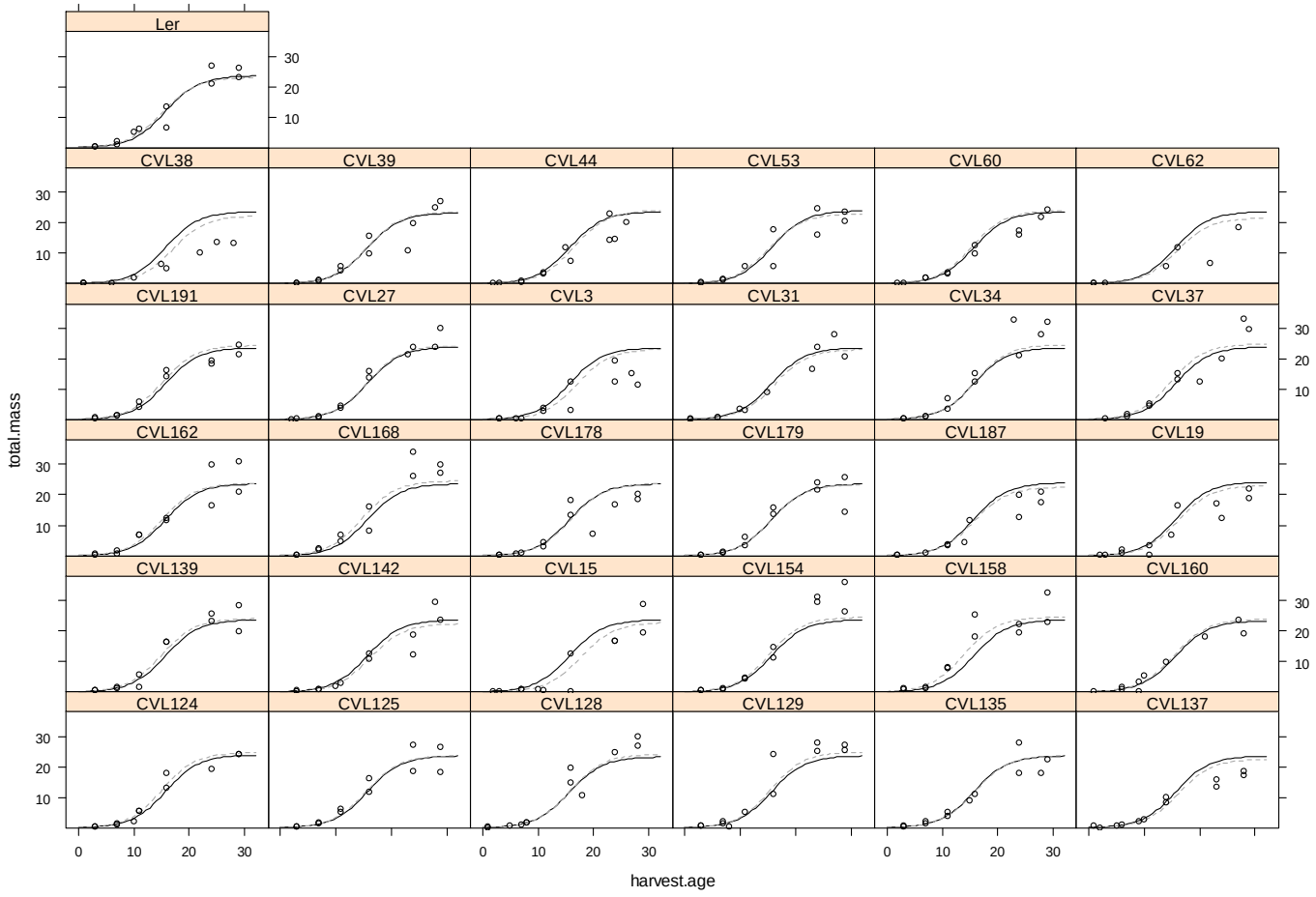

Figure 6. Observed total mass (circles) versus harvest age and the final model from the logistic model with fixed effects (solid lines) and RILs effects (dotted lines) for plants grown in 30 mm diameter pots.

Number of leaves

Pot volume had a significant effect on the asymptotic number of leaves produced (*Asym*: $F_{1,968} = 873$, $p < 0.0001$). Thus, the plants produced more leaves in bigger pots although the difference was small with on average 8.54 leaves (CI 95%: 8.35 – 8.72) in 20 mm pots and on average 9.42 leaves (CI 95%: 9.21 – 9.58) in 40 mm pots (averages calculated from the estimates table of the final model Table 2). The parameter *lrc,* which controls the rate at which the asymptote is approached was not affected by pot volume ($F_{1,968} = 0.170$, $p = 0.65$). Thus although the plants accumulate mass at a faster rate in bigger pots, they do not change the rate of leaf production (Table 2). The parameter *R0* was unaffected by pot volume ($F_{1,969} = 3.43$, $p = 0.0644$) which was expected as all plants start with two leaves regardless of pot size.

Table 2. Estimates of fixed effects from the final asymptotic model (non linear mixed-effects model) fitted to the leaves number produced against plant age. Random, i.e. line effects were retained for *Asym.*

	Value	Std.Error	DF	t-value	p-value
Asym.(Intercept)	4.98	0.650	970	7.66	<.0001
Asym.log(pot.vol)	0.619	0.0917	970	6.74	<.0001
R0	-0.621	0.212	970	-2.93	0.0035
lrc	-1.92	0.0692	970	-27.8	<.0001

A large effect of line was observed for the two parameters *Asym* and *lrc* (*Asym*: $\chi^2 = 159$, $p < 0.0001$ and *lrc*: $\chi^2 = 46.2$, $p < 0.0001$). There was a variation among the lines in the asymptotic number of leaves produced (*Asym*) and the rate at which the asymptote was approached (*lrc*). There was again no effect of line identity on the initial number of leaves (*R0*: $\chi^2 = 1.73$, $p = 0.189$). *R0* was not affected by line, which was also expected as all plants start with two leaves regardless of line. It was therefore removed from the final model. Parameter estimates for the final model is shown in Table 2 and plots of the data vs. the predicted values from the final model in Figure 7.

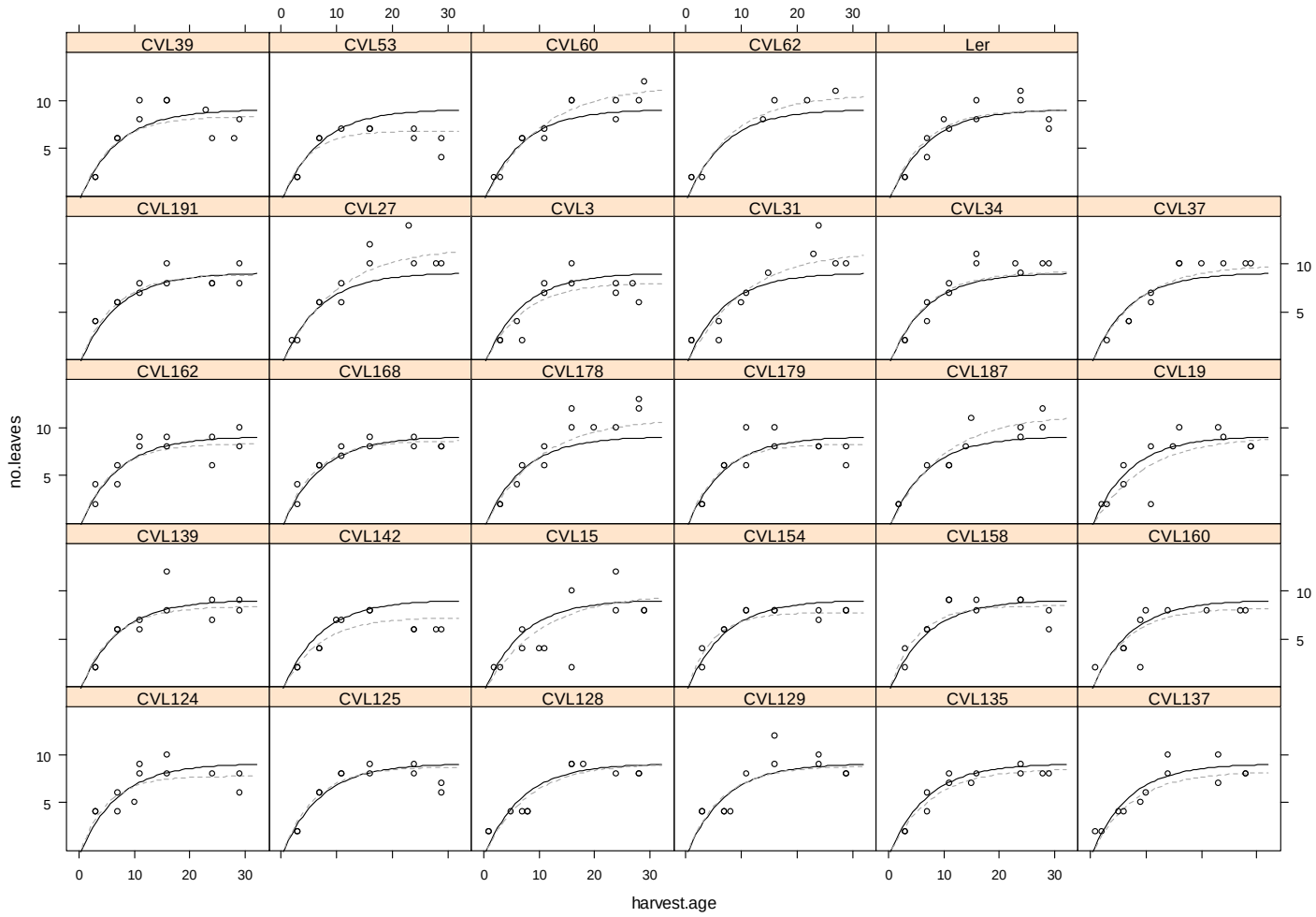

Figure 7. Observed number of leaves (circles) versus harvest age and the final model from the asymptotic model with fixed effects (solid lines) and RILs effects (dotted lines) for plants grown in 30 mm diameter pots.

Bolting age

The observed bolting age was not significantly affected by the sown seed mass ($F_{1,26}$ = 0.650, p = 0.427, Table 3). The small seeded lines therefore initiated flowering at the same time as the bigger seeded lines. This result is in disagreement with the data found previously (Figure 4) by Alonso-Blanco et al. (1999) where small-seeded lines flowered later. Flowering initiation was not affected by the *erecta* mutation ($F_{1,26}$ = 0.499, p = 0.486, Table 3). Pot volume did not affect flowering initiation ($F_{1,56}$ = 0.0994, p = 0.754, Table 3), thus the plants initiated flowering at the same time regardless of pot volume. All interactions were non significant (Table 3).

Table 3. Anova table from the linear mixed-effects model of the bolting age with sown seed mass, pot volume and *erecta* mutation fitted as fixed effects. Line was treated as a random effect.

	numDF	denDF	F-value	p-value
(Intercept)	1	56	1609	<.0001
sown.seed.mass.est	1	26	0.650	0.427
log(pot.vol)	1	56	0.0994	0.754
erecta	1	26	0.499	0.486
sown.seed.mass.est:log(pot.vol)	1	56	0.0111	0.916
sown.seed.mass.est:*erecta*	1	26	0.0257	0.874
log(pot.vol):*erecta*	1	56	0.0137	0.907
sown.seed.mass.est:log(pot.vol):*erecta*	1	56	0.386	0.537

As pot size was not significant, we then compared mean bolting age per line with the time to reach half-final mass (*Xmid*) and the estimated asymptotic mass (*Asym*) from our logistic model. Bolting age and *Asym* were uncorrelated (r = -0.34, p = 0.0645, Figure 8a). Although the significance is marginal, the direction of the correlation is different from that predicted by the traditional model i.e. it is negative. This negative correlation between bolting age and *Asym* might imply a cost of shunting matter from the vegetative parts to the reproductive parts for the large-seeded lines, which have a larger vegetative biomass to translocate. Therefore bolting later does not allow the plant to achieve a higher total mass or accumulate more resources as the *traditional flowering model* predicts (Mitchell-Olds 1996). However, *Xmid* and bolting age were correlated (r = 0.37, p = 0.044, Figure 8b). The average bolting time is 14.42 (CI 95%: 12.54 – 16.31) days while the average value of *Xmid* is 14.45 (CI 95%: 12.46 – 16.45) days. Therefore the flowering initiation coincides closely with *Xmid,* i.e. with the inflection point of the growth curve. This can be seen more clearly in Figure 9, where the bolting time and *Xmid* are plotted on the same graphic.

Finally we compared mean bolting age per line with the estimated number of leaves at bolting and the estimated mass at bolting from our logistic model. Bolting age and estimated number of leaves at bolting were positively correlated (r = 0.71, p < 0.0001, Figure 8c). Therefore plants which bolted later had more leaves. Bolting age and estimated mass at bolting were also positively correlated (r = 0.88, p < 0.0001, Figure 8d), thus the vegetative

mass accumulated at bolting is higher when the plants flower later, but critically the asymptotic mass is not higher.

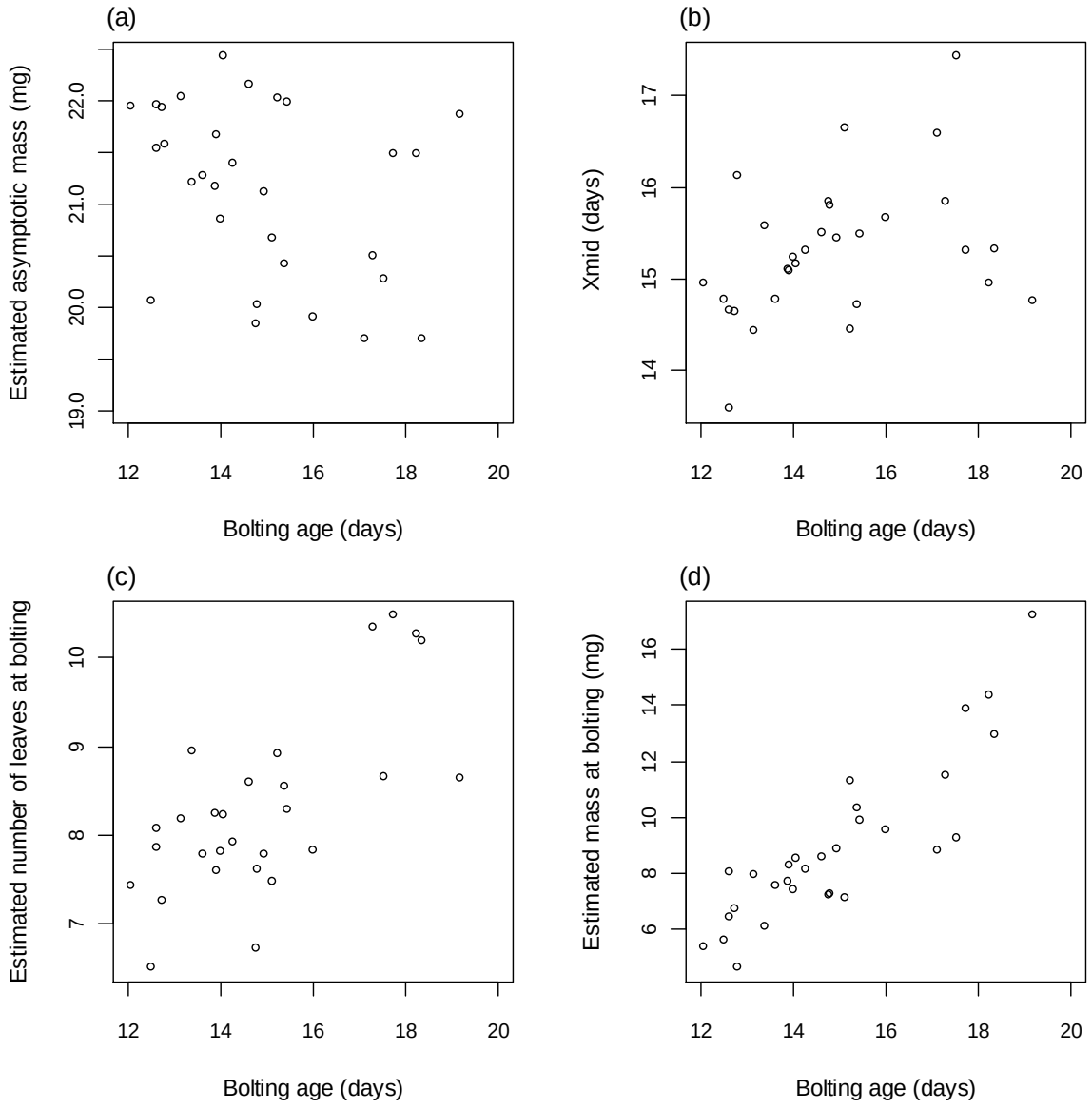

Figure 8. Estimated asymptotic mass versus bolting age (a), *Xmid* (the inflection point of the logistic growth curve) versus bolting age (b), estimated number of leaves at bolting versus bolting age (c) and estimated mass at bolting versus bolting age (d). Each point represents a single mean value per line averaged over all pot sizes and individuals.

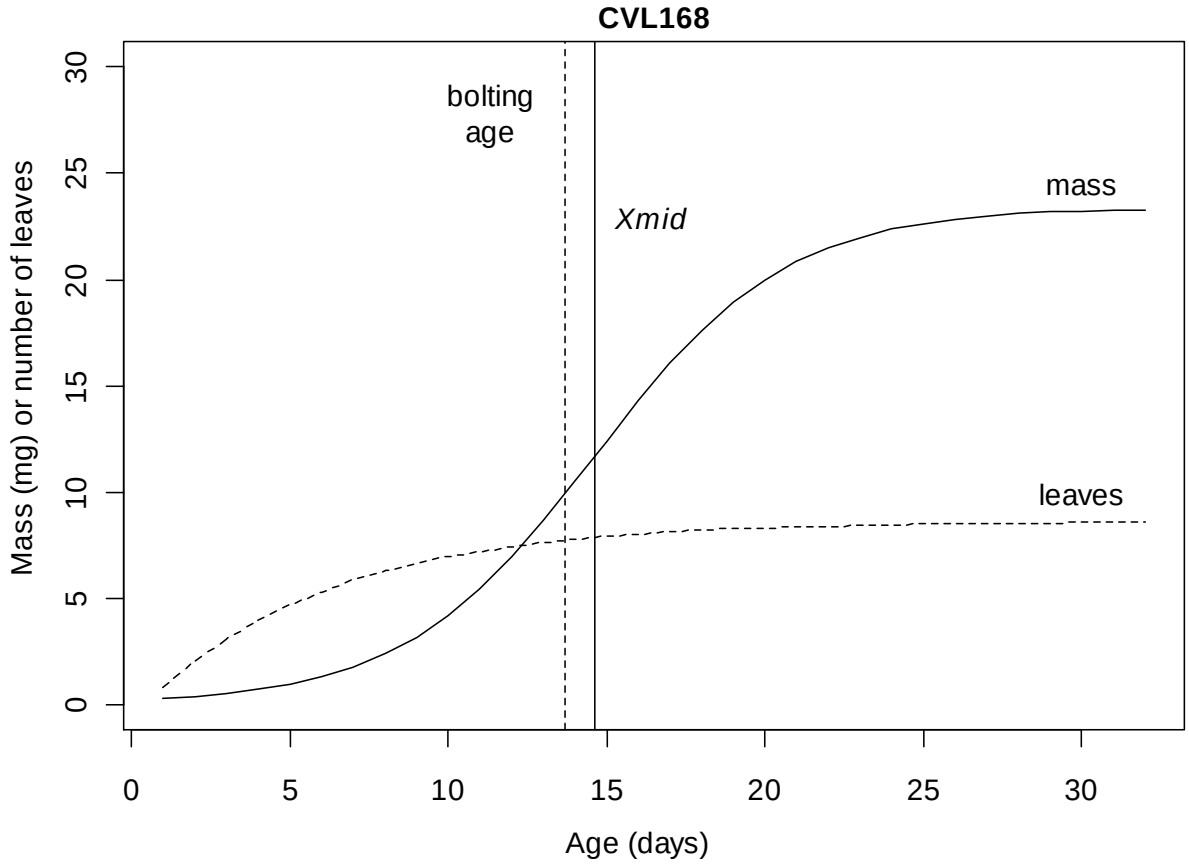

Figure 9. Model predictions from the non-linear regressions in the 30 mm diameter pots for the line CVL 168. The predicted number of leaves through time is represented by the dotted curve. The total mass through time is represented by the solid line. The solid vertical line is the inflection point of the total mass curve (*Xmid*). The dotted vertical line id the observed age. Notice that *Xmid* and bolting age correspond closely. There is little further leaf production after bolting but substantial further mass accumulation.

Total mass and number of leaves at bolting

To see whether there was further leaf production following bolting we calculated the proportion of the final leaf number (*P(FLN)*) produced at bolting as:

$$P(FLN) = \frac{\text{number of leaves on bolting day}}{\text{estimated asymptotic number of leaves}}$$

This information says how far advanced in leaf production the plant is at the observed bolting time. In order to see whether the plant activity was still going on despite of the end of leaf production, the proportion of total mass accumulated (*P(TM)*) at bolting was also analysed as:

$$P(TM) = \frac{\text{estimated mass on bolting day}}{\text{estimated asymptotic mass}}$$

The proportion of final leaf number at bolting (*P(FLN)*) was not affected by seed mass ($F_{1,24}$ = 0.034, p = 0.855, Table 4) or the *erecta* mutation ($F_{1,24}$ = 0.402, p = 0.532, Table 4). *P(FLN)* was also not significantly different between pot sizes ($F_{1,82}$ = 0.0805, p = 0.777, Table 4), i.e. the plants achieved the same proportion of final leaf number regardless of pot size. The plants were mainly at the end of their leaf production at bolting having on average 94.34 % (CI 95%: 92.08 – 96.61) of final leaf number on the bolting day. Thus when bolting was initiated, there was little further leaf production, indicating a hard switch from vegetative to reproductive growth as Cohen (1971) predicted in his model (see *Introduction*).

Table 4. Anova table from the linear mixed-effects model of the proportion of the asymptotic leaf number (*P(FLN)*) achieved at bolting with sown seed mass, pot volume and *erecta* mutation fitted as fixed effects. Line was treated as a random effect.

	numDF	denDF	F-value	p-value
(Intercept)	1	52	20608	<.0001
sown.seed.mass.est	1	24	0.034	0.855
log(pot.vol)	1	52	1.48	0.229
erecta	1	24	0.402	0.532
sown.seed.mass.est:log(pot.vol)	1	52	0.464	0.499
sown.seed.mass.est:*erecta*	1	24	0.124	0.728
log(pot.vol):*erecta*	1	52	0.085	0.771
sown.seed.mass.est:log(pot.vol):*erecta*	1	52	0.972	0.329

The proportion of total mass accumulated at bolting (*P(TM)*) was not affected by seed mass ($F_{1,26}$ = 0.0336, p = 0.856, Table 5) or the *erecta* mutation ($F_{1,26}$ = 1.48, p = 0.234, Table 5). Interestingly, the proportion of total mass accumulated at bolting was only 43.17 % (CI 95%: 36.15 – 50.19) differed significantly between pot sizes ($F_{1,56}$ = 340, p < 0.0001). The plants had accumulated a lower fraction of their final mass at bolting in bigger pots : 35.8 % (CI 95%: 34.1 – 37.5) in 40 mm pots and 58.3 % (CI 95%: 56.6 – 60.0) in 20mm pots. Pot size has a significant effect on *P(TM)* because it significantly affected *Xmid* but not bolting age. Thus plants initiated flowering at the same time regardless of pot volume, but the time to achieve half this mass (*Xmid*) increased with pot volume.

Table 5. Anova table from the linear mixed-effects model of the proportion of total mass accumulated (*P(TM)*) when bolting was initiated at with sown seed mass, pot volume and *erecta* mutation fitted as fixed effects. Line was treated as a random effect.

	numDF	denDF	F-value	p-value
(Intercept)	1	56	349	<.0001
sown.seed.mass.est	1	26	0.0336	0.856
log(pot.vol)	1	56	340	<.0001
erecta	1	26	1.48	0.234
sown.seed.mass.est:log(pot.vol)	1	56	0.0024	0.961
sown.seed.mass.est:*erecta*	1	26	0.0491	0.826
log(pot.vol):*erecta*	1	56	0.354	0.554

If the rosette (e.g. vegetative part) stops growing at bolting as Mitchell-Olds model predicts (Mitchell-Olds 1996) then the proportion of final mass achieved at bolting would be 100 %. However we found that the proportion of final mass achieved at bolting is only around 44 %. Therefore roughly 50% of the total mass accumulated by a plant occurs after bolting is initiated and rosette growth stops. This means that the rosette is still physiologically active after rosette growth stops as the remobilization of carbon from the vegetative parts cannot be the unique source of matter accumulation for the reproductive part.

DISCUSSION

We grew individual *Arabidopsis thaliana* plants from a recombinant inbred population in such a way that belowground resources limited growth and provided three levels of resource availability (pot volumes). By fitting models to our data, we estimated the number of leaves and the total mass of the plants when flowering was initiated. We also calculated several parameters as the asymptotic total mass and the asymptotic number of leaves for each plant.

On average, plants were bolting at the same time regardless of pot size which is the contrary of what expected by the Iwasa model (2000) predicting later flowering in a favourable growth environment (see *Flowering rules*). Perhaps our pot sizes were perhaps not sufficiently large to observe this flowering delay. We observed little sensitivity with regard to pot size within our experiment. Experimental studies reported that earlier flowering was

observed in better growing conditions (Bagnall 1991, Sugiyama and Hirose 1991, Pigliucci and Schmitt 1999). These different results might reflect a more complex process involved in the flowering initiation or species differences in responses to environment. Flowering too late, or too early can both have consequences and costs for the plants. Earlier flowering is favoured when there is high mortality of reproductive individuals (Kozlowski and Wiegert 1986, Kudoh et al. 2002) and may be advantageous because it allows escaping from local events such as harvest of agricultural areas, and avoiding summer drought (Mitchell-Olds 1996). Probability of survival is therefore the major cost of delayed flowering (Kozlowski 1992). According to the nature of the environment, results are different for bolting initiation. Our study is focused on the bolting decision for annuals (the decision to switch from the vegetative to the reproductive phase) and whether plants make the switch at an optimal time in absence of competition and with a stable environment (no drought, no habitat destruction). This may explain the difference in the results found in the other studies above. We aimed to understand the bolting initiation in a short term basis, not long term basis. Pigliucci and Schlichting (1996) also observed that some of the *Arabidopsis thaliana* ecotypes with slower growth rate flowered earlier. They measured the growth rate as the total height of the stem divided by the difference between senescence time and bolting time. We have seen that initiating bolting earlier might lead to a slower subsequent growth rate because the plant did not reach yet its maximal potential growth rate (see *Introduction*). So according to the *dynamic optimisation model* (Iwasa 2000), the results found by Pigliucci and Schlichting (1996) should be interpreted as a slower growth rate because of an early flowering and not the contrary.

The bolting age (flowering initiation) coincided closely with *Xmid*, i.e. with the inflection point of the growth curve. *Xmid* was also greater with increasing of pot sizes but not bolting age which explained why plants produced proportionally more of their final mass in lower pot volume at bolting. Our plants were mainly at the end of their leaves production at bolting

for all pot volume meaning that the vegetative part stopped growing from the bolting initiation. Hence, our results shows the clear switch from vegetative to reproductive growth already observed in may annual plants (Cohen 1971, Schaffer 1977, King and Roughgarden 1982, Iwasa 2000). On average 50% of the total mass accumulated by a plant occurred after bolting was initiated and rosette growth stopped. The plant stops investing in vegetative part, no gain of mass, but no loss either (Iwasa 2000). Our plants were still actively producing matter and not simply using the vegetative parts to remobilize matter into reproductive parts. This contradicts the *traditional flowering model* (Mitchell-Olds 1996) and supports the *dynamic optimisation model* (Iwasa 2000).

Bolting age and asymptotic total mass were not correlated, indicating that bolting later did not allow the plant to accumulate more resources in total. This result contradicts again the *traditional flowering model* (Mitchell-Olds 1992, 1996) which predicts the later a plant initiates flowering, the greater will be its fecundity. An early-flowering plant would have less resource accumulated to shunt into its reproductive part. It is commonly believed that longer vegetative growth allows annual plants to increase their size and consequently their seed output (Kudoh et al. 2002). If later flowering is so advantageous, the maintenance of early flowering is explained by cost of delaying flowering that counterbalances the benefit of longer vegetative growth resulting in the evolution of optimal flowering time (Law et al. 1977, Rathcke and Lacey 1985, Kudoh et al. 2002). Therefore, flowering time (age at reproduction) and size at reproduction are considered as important fitness components of annual plants (Stearns 1992, Mitchell-Olds 1996, Kudoh et al. 2002) and can lead to different life history strategies (Climent et al. 2008).

Seed mass did not affect total mass, number of leaves, bolting age, the proportion of total leaf number at bolting or the proportion of final mass accumulated at bolting. We do not find the relationship previously obtained by Alonso-Blanco et al. (1999), where small-seeded lines flowered later and had higher total seed outputs.

Does the rosette become inactive at bolting? Complete remobilization of vegetative part vs. actively photosynthetic rosette.

The attention was previously focused on the facts that natural selection favors plants that flower early and attain large size at first reproduction (Mitchell-Olds 1996) and that larger size is often associated with greater fecundity in annual plants (Mitchell-Olds 1992). Therefore a late flowering plant leads to a longer vegetative growth period that promotes the accumulation and allocation of more resources to seed production, whereas early flowering is selected in environments with a short or unpredictable growing season (Simpson and Dean 2002, Komeda 2004, Roux et al. 2006). It was believed that being an early flowering is a disadvantage for the quantity of offspring produced because it gives less time to accumulate minerals, but this is only true if the vegetative part stops being active when is bolting as expected in the *traditional flowering model* (Mitchell-Olds 1996). The flowering stage would mean a complete stop of photosynthesis activity or uptake from the roots. The data obtained from our study showed plants are still active which is supported by the study from Waters et al. (Waters and Grusak 2008). They showed that plants do continue to actively translocate minerals from the soil after flowering is initiated. Waters and Grusak (2008) found that the total mineral content of shoot tissues continued to increase even as seeds were maturing. They did observe remobilization but according to them it is unlikely that 100% of the mineral loss from leaves went to seeds. Therefore the remobilization of minerals from *Arabidopsis* leaves is not absolutely required for seeds to acquire minerals. This active uptake of minerals is then very important to take in account especially for understanding plant growth and plant's decision like bolting initiation in various conditions. A plant which flowers earlier is not anymore bound to store less minerals in its seeds, because it does not need to be stored in the plant, it can be translocated directly from the roots to the seeds, saving of tissues and building costs. What happens in reality is probably a mix between remobilization and

continued active uptake, as it seems unlikely that remobilization efficiency would be 100 %, or that plants are unable to transfer any of their accumulated resources.

When does Arabidopsis thaliana initiate bolting? The age rule vs. the tactical response to environment.

Plants show a great phenotypic plasticity in growth. The numbers and relative sizes of organs often change with local environmental conditions (Iwasa 2000). Here the RILs we used enabled us to study the bolting initiation of heterogeneous lines in different environments. The trait which was highly constrained was the timing of bolting initiation. In all pot sizes, the timing of bolting was the same. This result rather supports the *traditional flowering model*. The trait where the plants showed a great plasticity was the proportion of mass achieved at bolting. It appeared that bolting initiation was not constrained as much as the *traditional flowering model* predicted or as the relationship found by Alonso-Blanco et al. (1999) Figure 4. The starting mass of a plant (e.g. its seed mass) or the mass at bolting (size at reproduction) or the age at bolting initiation (age at reproduction), have no effect on the seed output.

The strong positive relationship between number of leaves produced at flowering and age at flowering led to the concept of a highly constrained switch. The number of leaves is viewed as very good estimate of flowering time (Mitchell-Olds 1996, Alonso-Blanco et al. 1998, Ungerer et al. 2002, El-Lithy et al. 2004, Passardi et al. 2007). This could be explained in two ways. First, plants could use an age rule and initiate flowering at a particular number of leaves. The switch would be determined by the number of leaves. Secondly, plants do not use an age rule and the number of leaves could be a result of bolting initiation. We saw that the rosette stops growing at bolting initiation, so the number of leaves would be determined simply by the number of leaves achieved at this particular moment. It is normal to find a high

correlation between number of leaves produced at flowering and age at flowering because plants, but the causality is still unclear.

The bolting age and the inflection point of the curve (*Xmid*) where half of the total mass is accumulated are correlated. The same problem of causality emerges here. This could be explained in two ways again. First, if plants use an optimum strategy, they would initiate bolting when the accumulated mass reaches 50 % because afterwards the intrinsic growth rate is bound to decrease. The mass accumulated would determine the switch. Secondly, plants do not use an optimum strategy and the mass accumulated at bolting is determined by the bolting age itself. But this second option would be very unlikely because the majority of the lines showed a switch around 50 %. The likelihood to see this general pattern appearing by chance would be quite limited.

Our study supported mainly the Iwasa model (2000). The only difference was about the bolting age which occurred at the same time regardless pot size in our experiment. The *Arabidopsis* plants thus seem to possess the ability to "sense" the environment by initiating bolting when their intrinsic growth cannot be maximized anymore. It seems that delaying flowering does not lead to higher reproductive mass when a plant has a fixed amount of resources available in the environment. We did not observe a rigid "clock"-like strategy but rather an optimal solution involving sensing the environmental cues.

SUPPLEMENTARY INFORMATION

Appendix S1: Information about the 32 lines selected for the study. The two accessions L*er* and Cvi are the parents. The 30 remaining recombinant inbred lines are derived from reciprocal crosses between the two parents.

NASC	RIL Koornneef	Published Seed Mass (*) [mg]	Sown Seed mass (**) [mg]	*erecta* mutation
N8581	L*er*	0.0193	0.0202	1
N8580	Cvi	0.0351	0.0348	0
N22002	CVL3	0.0162	0.0129	1
N22014	CVL15	0.0145	0.0193	0
N22018	CVL19	0.0251	0.0263	1
N22026	CVL27	0.0275	0.0270	1
N22030	CVL31	0.0295	0.0334	0
N22033	CVL34	0.0236	0.0297	0
N22036	CVL37	0.0325	0.0399	0
N22037	CVL38	0.0150	0.0188	0
N22038	CVL39	0.0202	0.0258	0
N22043	CVL44	0.0242	0.0285	0
N22051	CVL53	0.0327	0.0310	1
N22057	CVL60	0.0286	0.0393	1
N22059	CVL62	0.0190	0.0224	0
N22094	CVL124	0.0274	0.0252	1
N22095	CVL125	0.0200	0.0214	0
N22098	CVL128	0.0273	0.0274	0
N22099	CVL129	0.0243	0.0268	0
N22105	CVL135	0.0327	0.0348	1
N22107	CVL137	0.0302	0.0314	0
N22109	CVL139	0.0217	0.0231	0
N22112	CVL142	0.0315	0.0318	1
N22124	CVL154	0.0317	0.0323	0
N22128	CVL158	0.0373	0.0411	1
N22130	CVL160	0.0361	0.0402	1
N22132	CVL162	0.0256	0.0221	1
N22138	CVL168	0.0334	0.0299	0
N22148	CVL178	0.0207	0.0226	1
N22149	CVL179	0.0223	0.0243	1
N22156	CVL187	0.0183	0.0192	1
N22160	CVL191	0.0280	0.0257	1

(*) Source: Alonso-Blanco et al., 1999.

(**) Source: Arabidopsis center (TAIR).

Appendix S2: Relationship between the two alternative parameterisations of the logistic model used in this chapter.

The expected mass *M* after time *t* according the logistic growth curve from Hunt (1982) is:

$$M = \frac{KS\exp(\alpha t)}{K + S(\exp(\alpha t) - 1)} \quad \text{(eqn 1)}$$

where *K* is the maximum size the plant can attain, *S* the seed size (i.e. the initial plant size) and α is the intrinsic growth rate. The expected mass *y* after time *x* according to the simple logistic model from Pinheiro and Bates (2000) is:

$$y(x) = \frac{Asym}{1 + \exp[(Xmid - x)/scal]} \quad \text{(eqn 2)}$$

where *Asym* is the estimated asymptotic mass of the plant, *Xmid* is the inflection point of the curve and *scal* is a scale parameter of the growth. The definition of *scal* will become clearer after the conversion of the equation (1) into equation (2). We know that $K = Asym$. They both represent the maximum plant size. Now, we start from the equation 1 and convert it into equation 2.

$$M = \frac{KS\exp(\alpha t)}{K + S(\exp(\alpha t) - 1)} \quad \text{(eqn 1)}$$

First we simplify the equation (1) by multiplying the denominator by $\frac{1}{S\exp(\alpha t)}$:

$$M = \frac{K \times S\exp(\alpha t) \times (1/S\exp(\alpha t))}{[K + S(\exp(\alpha t) - 1)] \times (1/S\exp(\alpha t))} \quad \text{(eqn 3)}$$

$$M = \frac{K}{[K/S\exp(\alpha t)] + \frac{S(\exp(\alpha t) - 1)}{S\exp(\alpha t)}} \quad \text{(eqn 4)}$$

$$M = \frac{K}{[K/S\exp(\alpha t)] + 1 - \frac{1}{\exp(\alpha t)}} \quad \text{(eqn 5)}$$

as $\frac{1}{\exp(x)} = \exp(-x)$ we obtain:

$$M = \frac{K}{\frac{K}{S}\exp(\alpha t) + 1 - \exp(-\alpha t)} \quad \text{(eqn 6)}$$

$$M = \frac{K}{1 + \exp(-\alpha t) \times \left[\frac{K}{S} - 1\right]} \quad \text{(eqn 7)}$$

$$M = \frac{K}{1 + \exp(-\alpha t) \times \left[\frac{K}{S} - 1\right]} \quad \text{(eqn 8)}$$

as $\exp(\ln(x)) = x$ for $x > 0$, we then have:

$$M = \frac{K}{1 + \exp(-\alpha t) \times \exp\left(\ln\left[\frac{K}{S} - 1\right]\right)} \quad \text{(eqn 9)}$$

as $\exp(x + y) = \exp(x) \times \exp(y)$, the denominator becomes:

$$M = \frac{K}{1 + \exp\left(\ln\left[\frac{K}{S} - 1\right] - \alpha t\right)} \quad \text{(eqn 10)}$$

$$M = \frac{K}{1 + \exp\left(\left(\ln\left[\frac{K}{S} - 1\right] \times \frac{1}{\alpha} - t\right)\alpha\right)} \quad \text{(eqn 11)}$$

Now we compare with the equation 2:

$$y(x) = \frac{Asym}{1 + \exp[(Xmid - x)/lrc]} \quad \text{(eqn 2)}$$

We can finally see the terms correspondences with:

$$Asym = K \quad \text{(eqn 12)}$$

$$Xmid = \frac{1}{\alpha}\ln\left[\frac{K}{S} - 1\right] \quad \text{(eqn 13)}$$

$$\text{and } scal = \frac{1}{\alpha} \quad \text{(eqn 14)}$$

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CHAPTER 4

Selection on flowering strategy in a multi-generational experiment with *Arabidopsis thaliana*

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ABSTRACT

We used experimental metapopulations to investigate how landscape characteristics may influence plant morphological traits associated with dispersal ability in fragmented landscapes. We selected a population of 19 recombinant inbred lines (RILs) of *Arabidopsis thaliana*, varying in seed mass and height. Ten of the selected lines carry the *erecta* mutation: these lines are expected to achieve poorer seed spatial dispersal. We manipulated both the degree of fragmentation (by using four patch sizes, and the rate of patch disturbance, by using two different patch disturbance regimes (static vs. dynamic). Dynamic landscapes are characterized by continual patch destruction and regeneration, while patches in static landscapes remain in place for several generations. To exclude the confounding effects of density and to confirm whether genuine selection had occurred, seeds sampled from generation 5 plants were then grown under standardized conditions (a single plant per pot). We measured the effects of five generations of selection on several plant traits (height, bolting age, flowering age, seed mass and seed production) in the 24 manipulated landscapes. The first time this was done (Fakheran 2009), there were indications of stress (e.g. leaf purpling). Thus, 30 individuals from each landscape were re-grown from seed under the same standardized conditions and these results are presented here. The disturbance regime strongly affected final plant height with plants being taller in dynamic landscapes. However, harvested seed mass, bolting age, flowering age and seed production all experienced similar selection pressures in static and dynamic landscapes, early flowering and small seeds being favoured. Although the mean trait values were not different in static and dynamic landscapes, less variation remained in dynamic landscapes for both flowering characteristics and seed mass. This loss of variation was not solely due to the loss of the *erecta* mutation from dynamic landscapes. It is therefore clear that dynamic landscapes exert stronger directional selection on plant traits than the static ones.

INTRODUCTION

Annual plants are specialists on disturbed areas and often persist by taking advantage of a fugitive niche by exploiting gaps from which they will be eventually excluded by perennial species (Turnbull et al. 2004). Frequent disturbance of the habitat allows constant creation of empty patches that annuals can exploit. In order to maintain the population, the seeds from annual plants must colonize these suitable patches. These patches potentially show different dynamics. If suitable patches are stable, i.e. remain in the same place year after year, limited dispersal ability is likely to be selected (Hastings 1983, Cheptou et al. 2008). In these patches where the density of individuals is likely to be high, competition ability will be selected. If suitable patches are ephemeral, i.e. they move around year after year, good dispersal ability will be selected for and competitive ability will be less selected as density is likely to be low. Therefore the traits annual plants exhibit are influenced by landscape characteristics.

Plant height, seed size, seed number and flowering time of plants are traits likely to affect dispersal and competitive ability. For example, characteristics of plant architecture such as plant height, strongly determine seed dispersion patterns (Wender et al. 2005). Taller plants are expected to disperse seeds further so that if suitable patches move around the landscape, taller plants are likely to be selected. In contrast, when suitable patches are stable, it might be better no to disperse and shorter plants are likely to be selected (Cheptou et al. 2008).

Seed size is also a trait which can determine seed dispersion patterns (Wender et al. 2005). One important element in the reproductive strategy of a plant is the partitioning of its seed output into many small seeds or a few large ones (Gadgil and Solbrig 1972, Smith and Fretwell 1974, Geritz et al. 1999). The model from Smith and Fretwell (Smith and Fretwell 1974) predicts that there will be a single optimum seed size that is evolutionarily stable (Lloyd 1987): individuals that produce seeds either smaller or greater than the optimum suffer

reduced fitness (Lloyd 1987, Geritz 1995). However, this model fails to explain why such a variation of seed sizes is observed in nature between species that share the same habitat (Geritz 1995, Rees and Westoby 1997, Turnbull et al. 1999).

Small-seed species are associated with good colonizing ability, i.e. enhanced dispersal (Rees 1995, Turnbull et al. 1999, Coomes and Grubb 2003, Turnbull et al. 2004), because if a seed size/number trade-off operates, small-seeded plants will produce more seeds (Smith and Fretwell 1974, Venable 1992, Turnbull et al. 1999, Nathan et al. 2002, Wender et al. 2005). However, many plants are reported to produce large seeds despite the advantages of small seeds (Turnbull et al. 1999). The most common explanation of the existence of large seeded-species is their superior competitive ability (Rees 1995, Turnbull et al. 1999, Coomes and Grubb 2003, Turnbull et al. 2004). Large seed size confers an advantage of higher seedling survival or growth (Weis 1982, Stanton 1984, Weller 1985, Marshall et al. 1986), greater success in emerging from deep burial (Stanton 1984, Weller 1985, Wulff 1986, Mazer 1987) and positive effects on germination (Venable 1992). Thus large-seeded species may have greater competitive ability (Wulff 1986, Rees and Westoby 1997, Chacon et al. 1998, Turnbull et al. 1999, Coomes and Grubb 2003) and/or an establishment advantage (Freckleton and Watkinson 2001, Leishman 2001, Dalling and Hubbell 2002, Turnbull et al. 2004). Thus, in more undisturbed landscapes large seeds may have an advantage as they are better competitors while in highly disturbed habitats small seeds may be selected because they are better colonizers.

In models, small and large seeds can both be maintained within a single habitat if one assumes extreme (i.e. infinite) asymmetric competition (Skellam 1951, Tilman 1994, Rees and Westoby 1997, Geritz et al. 1999). Asymmetric competition is an unequal sharing of resources as a consequence of larger individuals having a disproportionate competitive advantage over smaller ones (Freckleton and Watkinson 2001). In the case where asymmetry is infinite, a species with a particular seed mass would be totally unaffected by competition

with any species with a lower seed mass, no matter how small the size difference (Kinzig et al. 1999, Levine and Rees 2002, Turnbull et al. 2008).Such infinite asymmetry is biologically unfeasible (Kinzig et al. 1999) and relaxing the assumption of extreme asymmetry only allows coexistence of a small number of species (Adler and Mosquera 2000). Another explanation of the maintenance of very different seed sizes would be an equalising trade-off between seed mass and seed number (Schamp et al. 2008). Under size-symmetric competition, where resource capture is proportional to mass, the outcome of competition could be insensitive to whether species produce many small seeds or fewer large ones. Seed mass would therefore be a neutral trait subject to genetic drift (Dalling and Hubbell 2002). However, in model simulation, this equalising trade-off has shown not to be neutral indicating that some other stabilising mechanism is also required (Turnbull et al. 2008).

Another important element in the reproductive strategy for annuals is the correct timing of the reproduction (Simpson and Dean 2002, see Chapter 3). Early flowering may be advantageous allowing escape from local disturbances, for example when the suitable patches do not last long, or avoidance of deteriorating environments resulting from summer drought (Mitchell-Olds 1996). On the other hand, late-flowering plants may be better competitors by having more time to grow their vegetative part (e.g. large rosette), and hence out-competing their neighbours by shading them or taking up more resources (Chapter 3). Therefore, early flowering plants are selected in unpredictable or highly disturbed habitats, whereas in favourable environments late flowering plants tend to be selected (Iwasa 2000).

If differences in the typical disturbance regime of the habitat lead to different densities, then undisturbed habitats will often be associated with high density and vice-versa. This reinforces the links between competition ability and dispersal ability and would likely lead to correlations among traits (similar to r and K selection, Gadgil and Solbrig 1972). For example, in highly disturbed habitats individuals are characterized by tall height, production

of large quantities of small seeds and early flowering. In contrast, in less disturbed habitats where the density of individuals is much higher, plants would possess adaptations to strong intra-specific competition and limited dispersal. Individuals are therefore likely to be smaller; produce fewer but larger seed and flower later.

To understand the selection forces involved in the different strategies for dispersal we performed an experiment using natural genetic variation in the model plant *Arabidopsis thaliana* (Alonso-Blanco et al. 1999). *Arabidopsis thaliana* is a widespread annual weed of rocky places and disturbed sites, native to Europe and central Asia and naturalized in North America. Across this geographical range, it experiences a broad range of climatic conditions (Hoffmann 2002) and selective pressures (Mitchell-Olds and Schmitt 2006). In Western Europe it can be a long-term resident of stone walls and other sites (Mitchell-Olds and Schmitt 2006). The chosen genotypes were used in Chapter 2 and showed a seed size/number trade-off. Furthermore, this annual plant enables us to achieve several generation of selection in a reasonable amount of time to test different disturbance regimes.

We created artificial landscapes with islands of suitable habitat embedded in an unsuitable matrix to simulate islands of natural habitats in nature (Hanski 1999, Cook et al. 2002). We manipulated both the degree of fragmentation and the rate of patch disturbance by using two different patch disturbance regimes (static vs. dynamic). In static landscapes, suitable habitats remain suitable over several generations; but in disturbed or dynamic landscapes suitable habitat patches are only available for one generation. They are then destroyed and regenerated in new locations in the landscape (For more details see *Methods, Dynamic vs. static Landscapes*). We seeded the landscapes with nineteen recombinant inbred lines (RILs) of *Arabidopsis thaliana* differing dramatically in traits thought to be associated with dispersal and competitive ability; i.e. height, *erecta* mutation, seed size, seed number and bolting age (the *erecta* mutation is known to dramatically diminish plant height). We then measured the effects of five generations of selection on these same plant traits by growing them under

standardized conditions. The first time plants were grown under standardized conditions (Fakheran 2009) plants showed signs of water stress. We therefore regrew plants a second time and took more extensive measurements, including the bolting and flowering date of each individual and its seed mass and seed output.

Aim of the study

In this chapter, we aim to investigate the following questions:

1) We hypothesise that long-range dispersal will be selected in dynamic landscapes and short-range dispersal in static landscapes.

2) Because of the overwhelming effect of the *erecta* mutation on plant height, we expect a higher frequency of the *erecta* mutation in static than in dynamic landscapes after 5 generations of selection. As the *erecta* mutation has sometimes been shown to reduce growth rates (Mitchell-Olds 1996) it might be eliminated from all landscapes.

3) If small seeds disperse further, we expect to find smaller-seeded lines in dynamic landscapes and if large seeds confer a competitive advantage under high-density conditions, we expect to find larger-seeded lines in static landscapes (as densities were higher in static landscapes, in Fakheran 2009).

4) As seen in Chapter 1, the RILs present a perfect seed size/seed number trade-off when adult size is constrained by the environment. Therefore, if the smaller-seed lines are selected in dynamic landscapes we expect to find a higher output of seeds per plant. For the static landscapes we would expect a smaller seed output due to the presence of large-seeded lines.

5) If late flowering is competitive, then we might find late flowering individuals in static landscapes because of higher density (Fakheran 2009). If early flowering has an advantage in disturbed habitats, we expect to find early flowering lines selected in dynamic landscapes

MATERIAL AND METHODS

Plant Material

We selected a population of 162 recombinant inbred lines (RILs) of *Arabidopsis thaliana* (Alonso-Blanco et al. 1999). The RILs are derived from reciprocal crosses between the two pure lines Landsberg *erecta* (L*er*), obtained as a mutant (*er*) from an accession of northern Europe (Rédei 1962, 1992), and Cvi, an accession from the tropical Cape Verde Islands (Lobin 1983). The two parents L*er* and Cvi have, respectively, small and large seeds (L*er*: 1.93 mg ± 0.10; Cvi: 3.51 mg ± 0.08; mass per 100 seeds, mean ± 1 SD; Alonso-Blanco 1999). The range in the seed mass exhibited by the entire RIL population (1.45 - 3.73 mg per 100 seeds) is greater than the variation expressed by the two parents.

Lines carrying the *erecta* mutation typically have short and upright stems, round leaves, short petioles and pedicels, flowers clustered at the top of the inflorescence, short and wide siliques with blunt tips, a compact inflorescence and reduced height (phenotype curated by the Arabidopsis Biological Resource Centre (ABRC)). The reduced height is the most striking thing about plants carrying the *erecta* mutation. Thus plants carrying this mutation are expected to achieve poorer spatial dispersal of their seeds (*Introduction*). The presence of the *erecta* mutation has also been shown to reduce growth rates over a 15-day period (Mitchell-Olds 1996), although its presence does not affect final seed outputs (Chapter 1) or growth rates in our work (Chapter 3).

We selected 19 lines from the possible 162. Originally, 30 lines plus the two parents were selected to use in experiments investigating the seed size/number trade-off (Chapter 1). From these 30, we selected 17 plus the two parents to use in the landscape experiment described here. We selected these lines in such a way as to maintain the seed mass variation present in the original RIL population. The lines can inherit the mutation *erecta* from the L*er*

parent. Ten of the selected lines carry this mutation, the other nine not (Table 1). All seeds were obtained from The Arabidopsis Information Resource (TAIR).

Table 1. Information about the 19 lines selected for the study. The two accessions L*er* and Cvi are the parents. The 17 remaining recombinant inbred lines (RILs) are derived from reciprocal crosses between the two parents. (*) Source: Alonso-Blanco et al., 1998, (**) Source: Arabidopsis center (TAIR).

NASC	RIL Koornneef	Published Seed Mass (*) [mg]	Sown Seed mass (**) [mg]	*erecta* mutation	Short number
N22018	CVL19	0.0251	0.0263	1	1
N22026	CVL27	0.0275	0.0270	1	2
N22030	CVL31	0.0295	0.0334	0	3
N22033	CVL34	0.0236	0.0297	0	4
N22036	CVL37	0.0325	0.0399	0	5
N22038	CVL39	0.0202	0.0258	0	6
N22051	CVL53	0.0327	0.0310	1	7
N22057	CVL60	0.0286	0.0393	1	8
N22095	CVL125	0.0200	0.0214	0	9
N22098	CVL128	0.0273	0.0274	0	10
N22105	CVL135	0.0327	0.0348	1	11
N22107	CVL137	0.0302	0.0314	0	12
N22112	CVL142	0.0315	0.0318	1	13
N22128	CVL158	0.0373	0.0411	1	14
N22138	CVL168	0.0334	0.0299	0	15
N22149	CVL179	0.0223	0.0243	1	16
N22156	CVL187	0.0183	0.0192	1	17
N8581	L*er*	0.0193	0.0202	1	18
N8580	Cvi	0.0351	0.0348	0	19

One hundred seeds of each line were collectively weighed to give a sown seed mass estimate for each line. *erecta* and non-*erecta* lines did not differ significantly in their sown seed mass ($F_{1,17} = 0.091$, $p = 0.767$) and their ranges were similar (Figure 1A). Mean height values for each line are taken from Alonso-Blanco (1999), and represent the mean value from four individuals, grown in isolated pots. *erecta* and non-*erecta* lines differed dramatically in their height ($F_{1,17} = 51.07$, $p < 0.001$; Figure 1B). Among the lines there is a trade-off between seed size and seed number, such that under certain conditions, lines producing small seeds produce more (Chapter 1), however the total mass of seeds produced is not related to seed size (Chapter 1).

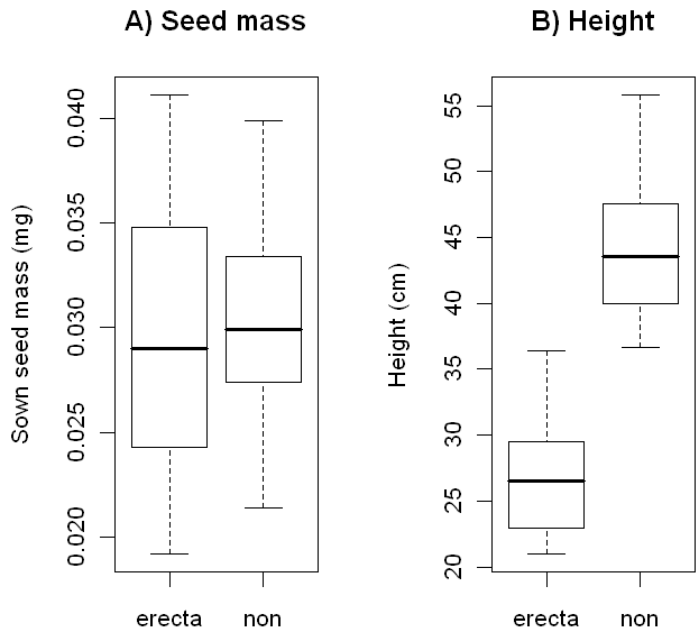

Figure 1. *erecta* and non-*erecta* lines have similar seed mass distributions (A), but they differ dramatically in their heights (B). Bold horizontal lines represent the median, boxes the interquartile range, and whiskers the maximum and minimum values (Fakheran 2009).

Landscapes

We set up a habitat fragmentation experiment using 24 landscapes with different degrees of fragmentation of the suitable habitat. The experiment was run for five generations in a glasshouse. Each landscape measured 90 × 64 cm, and consisted of patches in which *Arabidopsis* plants were allowed to grow (suitable habitat) and the matrix in which any plants growing were regularly removed (unsuitable habitat). The suitable habitat made up around 7 % of the total landscape (Table 2). After five generations individuals were sampled from each landscape and grown individually in a common garden experiment.

Table 2. Experimental design: Four different patch sizes provide different degrees of habitat fragmentation. The patch size was chosen to keep the total area of suitable habitat constant; however, it varies slightly with the number of patches.

Number of patches	Area of patch (cm^2)	Total suitable area (cm^2)	% Suitable area
2	240	480	8.3
4	100	400	6.9
8	52	416	7.2
16	25.5	408	7.1

Landscapes were constructed by filling a large tray (90 × 64 cm) with a mix of 50% soil and 50% sand. The patches were cylindrical slices of PVC tubing, cut to the same depth as the trays (70 mm). Patches were pushed into the soil so that their tops were level with the soil surface. The suitable habitat consisted of 2, 4, 8 or 16 patches. The patch size was chosen to keep the total area of suitable habitat constant. However, due to constraint of available material, the total area of suitable habitat varies slightly with the number of patches (Table 2). The four patch sizes provide different degrees of habitat fragmentation. There were six replicates of each level of habitat fragmentation making 24 landscapes in total. Patches were located within landscapes in a stratified random way. The landscapes were divided into four equally-sized quarters and patches were located in the following way: in 2-patch landscapes, only one patch was allowed in each of two randomly selected quarters, in 4-patch landscapes only one patch was allowed per quarter, in 8-patch landscapes two patches were allowed per quarter, and in 16- patch landscapes four patches were allowed per quarter; however the location of patches within quarters in all cases were selected at random. This minimized within- treatment variations.

Dynamic vs. static Landscapes

As well as the patch size treatments, we imposed two different patch disturbance regimes. In the first (which we call static) seeds which fall into the natal patch are returned to the surface of a new patch in the next generation. To do this, seeds were manually released from the

siliques (by gently shaking the plants by hand) once the plants were mature and seeds were ripe. All plant material was then measured and weighed. The surface layer of soil containing seeds was then scraped away from each patch and placed in a Petri dish (one per patch) and placed in a fridge for one week. During this time all remaining soil was removed from the existing patches and replaced with fresh soil made up in the same way as before (50% soil and 50% sand). Thus seeds which do not disperse away from their natal patch have a much higher chance of entering the next generation, although seeds which land in another patch, and not in the matrix, can also enter the next generation.

In the second patch disturbance regime (which we call dynamic) new Petri dishes (of the same number and size as the existing patches) were randomly placed around the landscape to collect dispersing seeds before plants began to flower. Seeds were then manually released from siliques in exactly the same way as for static landscapes. The new Petri dishes containing any dispersed seeds were removed and placed in the fridge for one week. All plants were removed and weighed and all patches refilled with fresh soil. In the dynamic landscapes, seeds falling back into the natal patch have no chance of entering the next generation. Seeds from static and dynamic landscapes were removed at the same time and placed in the same fridge together for the same length of time. Notice that, in static landscapes each patch maintains its identity through time (seeds taken from patch i are returned to the same patch i, but in dynamic landscapes a patch in generation t +1 can not be identified with any particular patch in generation t.

Initialising landscapes

In generation 1, landscapes were initiated by introducing seeds of each of the 19 lines in the following way. The 19 selected lines were counted and sown so as to obtain equal initial densities in landscapes containing different patch sizes. We sowed one seed per line into each

of the smallest patches, and 2, 4 and 8 seeds per line into each patch in the 8, 4 and 2-patch landscapes, respectively. Thus initially 16 seeds of each line were introduced into each landscape. Seeds were initially counted into eppendorf tubes and then kept in a cold room at 4 °C for one week to overcome seed dormancy and ensure uniform germination of different lines. In total 7296 seeds were sown in the first generation in 180 patches of our 24 landscapes (Fakheran 2009 for more details about the experiment set up: *Timetable* in Chapter 4). In the next four subsequent generations, plants were allowed to set seed naturally. Thus, in total five generations were conducted.

Standardized conditions

At the end of generation five, 77 seed pods from 77 different plants in each landscape were sampled, labelled and kept separately in a cold room at 4 °C for one week. These seeds were then grown under standardized conditions (one plant per pot) to exclude the confounding effects of density and to measure the effects of 5 generations of selection on plant traits. The 19 original lines (17 RILs + 2 parent lines) were also grown under these standardized conditions (4 replicates for each line). Pots were filled with the same 50% soil/ 50% sand mixture, used in the experiment. Presence of the *erecta* mutation was recorded for all surviving individuals by Masaki Kobayashi and Matthias Helling from Evolutionary Functional Genomics, Institute of Plant Biology, University of Zürich. Then, 30 individuals from the original 77 in each landscape were randomly selected. The final height of each individual was measured and seeds were collected and saved. The average seed mass of individuals within each landscape was estimated by weighing a single combined sample consisting of 150 seeds: 5 seeds from each of the 30 individuals (weighed on a microbalance).

The estimated seed mass for the original lines (17 RILs + 2 parent lines) obtained with this first standardized conditions experiment (Fakheran 2009) were much lower than the sown seed mass (Figure 2a).

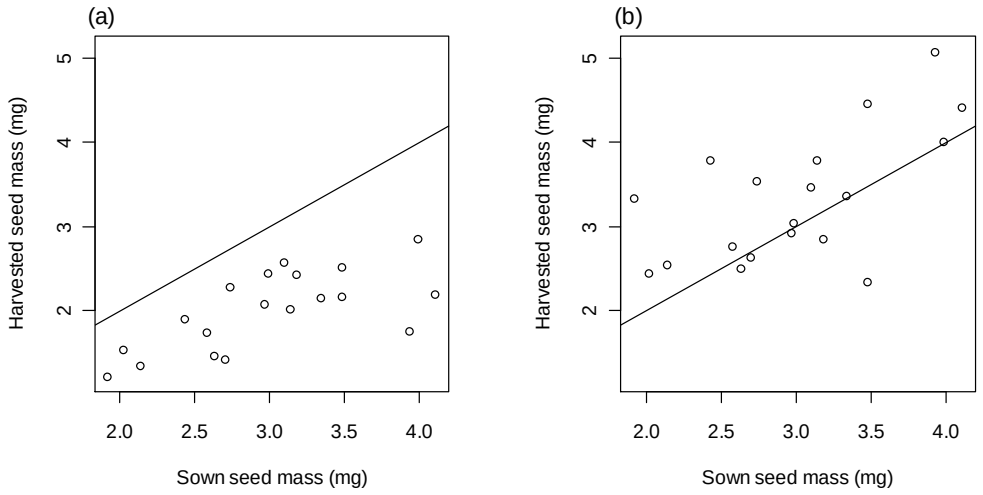

Figure 2. Comparison of the relationship between sown seed mass and harvested seed mass during the first standardized conditions (a) and the second standardized conditions (b).

This may be due to stress caused by over-watering in the early growth stages which led to the purpling of some individuals. Because we have saved seeds from each of the 30 individuals chosen randomly per landscape, we were able to re-grow individuals and repeat these measurements. We also grew four plants of each of the original lines. We then recorded detailed individual-level data from each plant. The following variables were measured on every plant from all landscapes and on the original lines.

1) *Germination day*
2) *Bolting day (day when the inflorescence is first seen) and flowering day (day where the first flower was observed)*
3) *Total number of siliques (seed pods)*
4) *Total number of seeds contained in two siliques*
5) *Weight of the seeds contained in two siliques*
6) *The final height of the main inflorescence*

The total number of siliques, the total number of seeds contained in two siliques and the weight of the seeds contained in two siliques allowed us to calculate the total number of seeds per plant (called harvested seed number) and the mass of 100 seeds (harvested seed mass) for each plant. The two seed pods (siliques) were always sampled from a similar location on each plant, normally the two ripe non-opened pods at the bottom of the inflorescence.

Statistical analysis

Here we present the analysis of bolting time, flowering time, final height, seed mass and seed number of individuals raised under the second standardized conditions. Because we measured multiple individuals from each landscape, we carried out the analysis using linear mixed-effects models using the function *lme* in the stats package R ((Pinheiro and Bates 2000); R Development Core Team 2007). Landscape identity (i.e. 1-24) was treated as a random effect and thus we could compare traits variation within and between landscapes. Patch area and the disturbance treatment (dynamic vs. static) were treated as fixed effects. Patch area was always log-transformed. The *erecta* mutation was included as a fixed effect for the final height analysis only. As seed mass was estimated from samples consisting of different numbers of seeds, we always standardized seed mass measurements to the mass of 100 seeds in milligrams (this also facilitates comparison with the data of (Alonso-Blanco et al. 1999)). It was apparent from preliminary data analysis that very often the variability differed in landscapes of different type. Thus we calculated the coefficient of variation for each trait in each landscape. The coefficient of variation (CV) is a good way to compare variation in samples with very different means and provides a measure of variation that is independent of the measurement units.

RESULTS

Final height

The height of four individuals from each of the 19 original lines grown under standardized conditions was measured. In the first standardized conditions (Fakheran 2009), the average height of the original lines was 21.9 cm (CI 95%: 19 – 24.8) while the mean height of plants in static landscapes was 20.1 cm (CI 95%: 19.7 – 20.5) and the mean height in dynamic landscapes was 35.5 cm (CI 95%: 35.2 – 35.8). In the second standardized conditions, the average height of the original lines is 22.81 cm (CI 95%: 20.49 – 25.13) while the mean height of plants in static landscapes is 20.2 cm (CI 95 %: 17.2 – 23.2) and the mean height in dynamic landscapes is 33.8 cm (CI 95 %: 32.5 – 35.0). These values are very similar to the ones found the first time this experiment was done (Fakheran 2009). This shows that the results are highly repeatable. Under the first standardized conditions, the lines were stressed, but obviously it had little effect on final height (pers. comm.. L. Turnbull).

As previously found (Fakheran 2009) final height was unaffected by patch size but the disturbance regime had a strong effect (Disturbance regime: $F_{1,20} = 247$, $p < 0.0001$; Patch size: $F_{1,20} = 0.017$, $p = 0.898$, Table 3).

Table 3. Anova table from the linear mixed-effects model of final plant height with the experimental treatments disturbance regime and patch size fitted as fixed effects. Landscape was treated as a random effect and forms the error term for the experimental treatments.

	numDF	denDF	F-value	p-value
Intercept	1	693	3925	<.0001
Disturbance regime	1	20	247	<.0001
Log(patch.size)	1	20	0.017	0.898
Disturbance regime : log(patch.size)	1	20	0.347	0.563

The height difference in the two disturbance regimes could be due to a change in two things: 1) a change in the frequency of the *erecta* mutation between static and dynamic landscapes, and/or 2) changes in individual height of both types (*erecta* and non-*erecta*). It is already known (Fakheran 2009) that the frequency of *erecta* differed between static and dynamic

landscapes. On average 8 % (CI 95%: 6.5 – 10.4) of individuals in dynamic landscapes carried the mutation, and in 3 of the 12 dynamic landscapes the *erecta* mutation was completely eliminated. For the static landscapes 44% (CI 95%: 37 – 51) of individuals carried the *erecta* mutation which was also close to the original frequency (52.6 %, Figure 3).

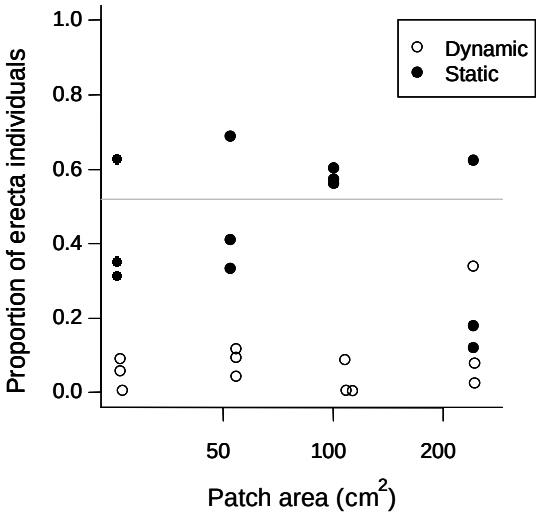

Figure 3. Frequency of the *erecta* mutation in each of 24 landscapes (dynamic vs. static) of different patch area. The frequency of the *erecta* mutation among the original lines is represented by the grey line (Fakheran 2009).

As well as selection for or against the *erecta* mutation, differences in average height among landscapes can also be due to changes in the average height of the individuals, both *erecta* and non-*erecta*.

The first standardized conditions (Fakheran 2009) showed that individuals carrying the *erecta* mutation were on average 6.23 cm (CI 95%: 3.35 – 9.11) shorter in static landscapes while non-*erecta* individuals were 10.83 cm (CI 95%: 8.4 – 13.23) shorter in static landscapes. The overall average height difference in static vs. dynamic landscapes was 15.39 cm (CI 95%: 14.81 – 15.98), which was greater than the difference in height among either *erecta* or non-*erecta* individuals. In consequence, the large overall difference in height between static and dynamic landscapes was due to both changes in the frequency of *erecta* and selection on the height of surviving plants.

In the second standardized conditions, we began by fitting a model with the experimental treatments disturbance regime, patch area, plus their interaction. However, similar to Fakheran (2009), only the disturbance regime was significant (Disturbance regime: $F_{1,20}$ = 247, p < 0.0001; Patch size: $F_{1,20}$ = 0.017, p = 0.898, Table 3). Their interaction was not significant. We then, fitted a second model containing the terms *erecta* mutation, disturbance regime plus their interaction. Both *erecta* mutation and disturbance regime were significant (*erecta*: $F_{1,691}$ = 188, p < 0.0001; disturbance regime: $F_{1,22}$ =538, p < 0.0001, Table 4). The individuals carrying the *erecta* mutation are on average 2.49 cm (CI 95%: 0.088 – 5.88) shorter in static landscapes while non-*erecta* individuals are 9.01 cm (CI 95%: 7.43 – 10.58) shorter in dynamic landscapes. The overall average height difference in static vs. dynamic landscapes is 13.56 cm (CI 95%: 11.82 – 15.30) which is again greater than the height difference between *erecta* and non-*erecta* individuals (Fakheran 2009). Thus these results are highly consistent with these of Fakheran (Fakheran 2009). This confirms that strong directional selection on height in dynamic rather than in static landscapes.

Table 4. Anova table from the linear mixed-effects model of final plant height with disturbance regime and *erecta* mutation fitted as fixed effects. Landscape was treated as a random effect.

	numDF	denDF	F-value	p-value
Intercept	1	691	8537	<.0001
Disturbance regime	1	22	538	<.0001
erecta mutation	1	691	188	<.0001
Disturbance regime : *erecta* mutation	1	691	14.8	0.0001

A comparison of the distribution of final height in static vs. dynamic landscapes together with the distribution of the original lines is shown in Figure 4. The original lines show a bimodal distribution with a main peak at 15-25 cm and a smaller peak of taller plants at 40-45 cm (Figure 4a) which is much less pronounced in the landscapes. The static landscapes still shows the first peak of the originals distribution, while the second smaller peak has almost vanished with very few tall individuals remaining (Figure 4b). In contrast, in the dynamic

landscapes the main peak is now centred on tall individuals with very few smaller individuals (Figure 4c).

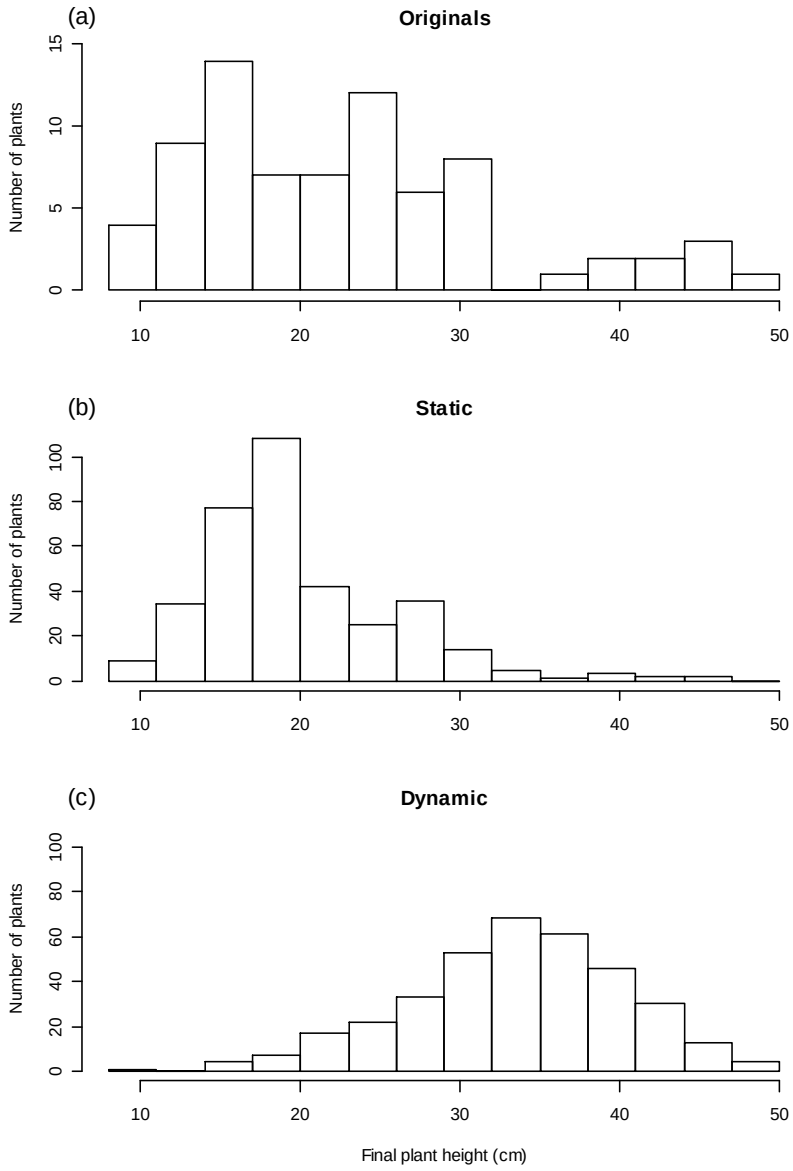

Figure 4. Histograms of final plant height (cm) for the original lines (a), and the two disturbance regimes: static landscapes (b) and dynamic landscapes (c). For the original lines there are four individuals from each of the 19 lines. In static and dynamic landscapes there are 30 individuals from each of the 12 landscapes respectively.

Bolting and flowering time

The bolting age and flowering age were calculated for each plant from the bolting day or flowering day minus the germination day. The results for the bolting age and flowering age analysis were very similar as the two variables are highly correlated (r =0.887, p <.0001, Pearson's product-moment correlation). Thus we present the histograms of both traits but only the analysis from bolting age is shown. Bolting age more accurately measures the timing of the plant's decision to switch to reproductive phase. Patch size and disturbance regime did not significantly affect age at bolting (Disturbance regime: $F_{1,20}$ = 1.035, p = 0.0.321; Patch size: $F_{1,20}$ = 2.56, p = 0.125, Table 5). Mean bolting age in static landscapes was 13.9 days (CI 95 %: 12.9 – 14.9) and 13.7 days (CI 95 %: 13.3 – 14.1) in dynamic landscapes. This similarity in the means (Figure 5) however, hides differences in the variation between static and dynamic landscapes (see Figure 5 and *Comparison of variation*). The means in the two disturbance regimes was similar to the original lines (14.22 days, CI 95%: 13.65 – 14.79), but the static landscapes (Figure 5b) had a greater variability than the dynamic ones (Figure 5c).

Table 5. Anova table from the linear mixed-effects model of bolting age with the experimental treatments disturbance regime and patch size fitted as fixed effects. Landscape was treated as a random effect. Bolting age is the time in days from germination to the first appearance of the inflorescence.

	numDF	denDF	F-value	p-value
Intercept	1	693	12507	<.0001
Disturbance regime	1	20	1.035	0.321
Log(patch.size)	1	20	2.56	0.125
Disturbance regime : log(patch.size)	1	20	2.62	0.121

Bolting age in original lines showed a bimodal distribution with a large peak at 13 days and a smaller peak of later-bolting lines at 17-18 days (Figure 5a). This bimodality is still apparent in the static landscapes but the second late-bolting peak has almost vanished in dynamic landscapes. It appears also that there may have been selection against very early flowering individuals (10-11 days) both in static and dynamic landscapes (Figure 5), although a few individuals flowering at 11 days remain in static landscapes (Figure 5b). The distribution of

flowering age (Figure 5d, e and f) is presented here to show the similarity with the bolting age distribution.

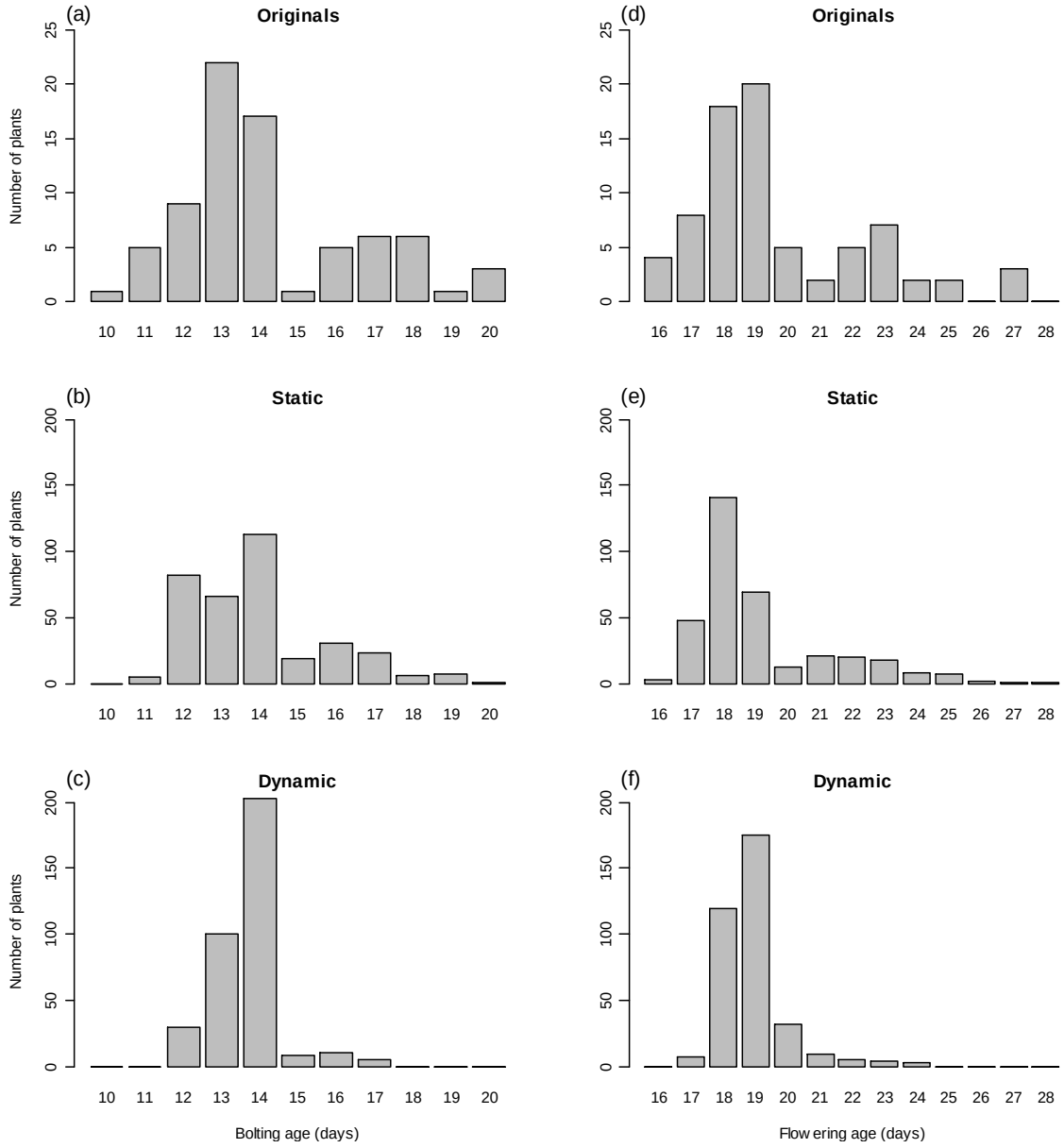

Figure 5. Histograms of bolting age (days) and flowering age (days) for the original lines (a, d), the static landscapes (b, e) and the dynamic landscapes (c, f). For the original lines there are four individuals from each of the 19 lines. In static and dynamic landscapes there are 30 individuals from each of the 12 landscapes respectively.

The clear bimodal distribution showed by the original lines was not due to the *erecta* mutation, as both lines carrying the *erecta* mutation (Figure 6b) and lines not carrying the

mutation (Figure 6a) have similar distributions with a small late-bolting peak at 17-18 days for the *erecta* individuals and 16-17 days for the non *erecta* individuals. Thus the loss of the *erecta* mutation in dynamic landscapes could not alone have caused the loss of the second late-bolting peak.

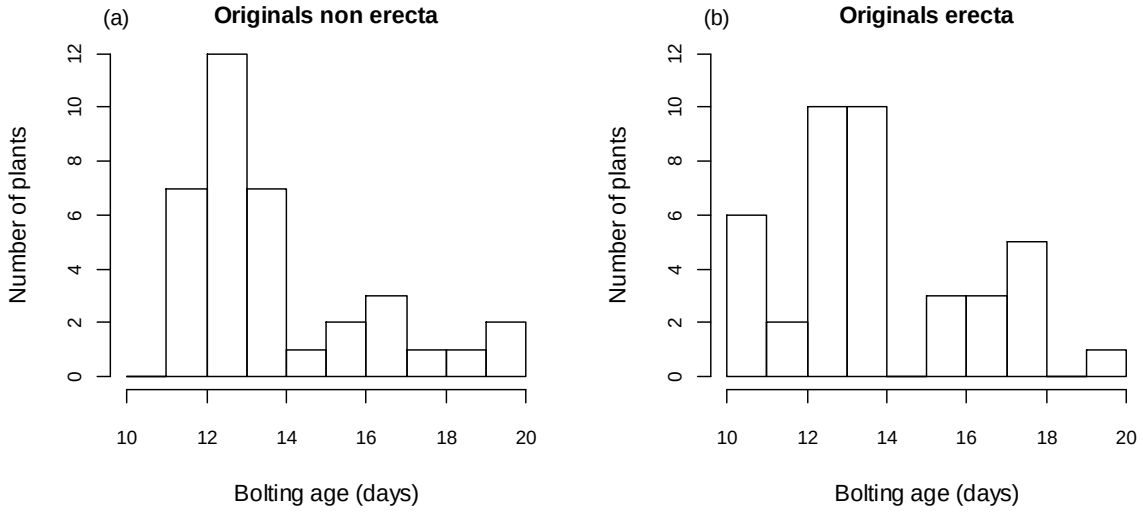

Figure 6. Histograms of bolting age (days) for the original non-*erecta* lines (a) and the original lines carrying the *erecta* mutation (b). Both show a bimodal distribution with small peak of late-bolting individuals.

Harvested seed mass

The seed mass (standardized to the mass of 100 seeds = *harvested seed mass*) was estimated as:

$$\text{harvested seed mass} = \frac{SMS}{NSS} \times 100$$

where *SMS* is the total mass of seeds from two siliques and *NSS* is the number of seeds in these two siliques on a given plant. We first compared the harvested seed mass to the sown seed mass (see *Biological material*). Harvested seed mass was considerably lower than sown seed mass in the first experiment under standardized conditions (Figure 2a), indicating possible effects of stress early in growth. The seed mass obtained in the second standardized conditions experiment were more consistent with the sown seed mass (Figure 2b) although they were generally higher than sown seed mass. This may be due to our pod selection when

we collected seeds. In order to have ripe seeds, we always collected pods from the bottom of the inflorescence. Siliques are not homogeneous along the inflorescence stem, as within a plant, seed size may vary according to the position of the seed within the plant or the inflorescence (Wulff, 1986). However, no signs of stress were noted as we were careful not to over-water seedlings in the early stages of growth. The harvested seed mass was not significantly affected by patch size or the disturbance regime (disturbance regime: $F_{1,20}$ = 2.98, p = 0.0999; patch size: $F_{1,20}$ = 0.117, p = 0.736, Table 6) although disturbance regime was marginally significant. The harvested seed mass in static landscapes is 2.65 mg (CI 95 %: 2.14 – 3.16) and in dynamic landscapes 2.39 mg (CI 95 %: 2.18 – 2.61). However, in both cases the mean is lower than the original lines (3.32 mg, CI 95%: 3.10 – 3.55, Figure 7a), indicating selection for smaller seeds in both disturbance regimes. Again, static landscapes (Figure 7b) had greater variability than the dynamic ones (Figure 7c). Thus dynamic landscapes appeared to have experienced much stronger directional selection. The fact that smaller seeded lines were selected under both disturbance regimes was not realized the first time plants were grown under standardized conditions. It is probably the large tail of large-seeded individuals in static landscapes that resulted in the significant difference between static and dynamic landscapes found before and the observed decline in seed mass over time in dynamic landscapes (Fakheran 2009).

Table 6. Anova table from the linear mixed-effects model of harvested seed mass with the experimental treatments disturbance regime and patch size fitted as fixed effects. Landscape was treated as a random effect.

	numDF	denDF	F-value	p-value
Intercept	1	651	1144	<.0001
Disturbance regime	1	20	2.98	0.0999
Log(patch.size)	1	20	0.117	0.736
Disturbance regime : log(patch.size)	1	20	0.110	0.744

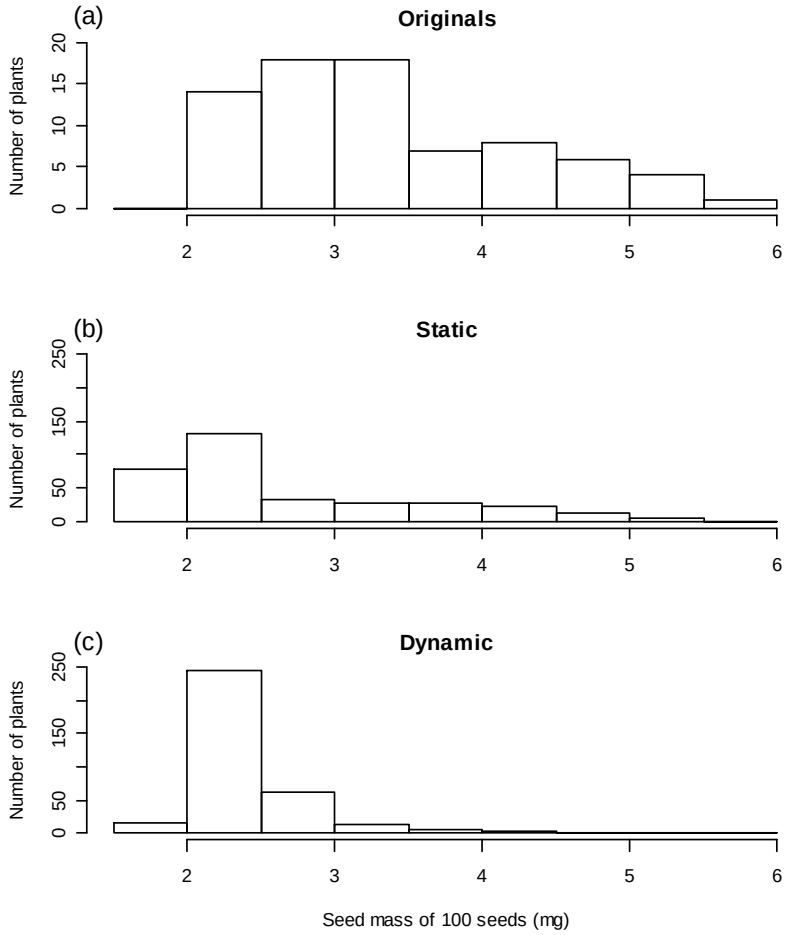

Figure 7. Histograms of harvested seed mass scaled to the mass of 100 seeds (mg) for the original lines (a), and the two disturbance regimes: static landscapes (b) and dynamic landscapes (c). For the original lines there are four individuals from each of the 19 lines. In static and dynamic landscapes there are 30 individuals from each of the 12 landscapes respectively.

Harvested seed number

The total number of seeds produced by a plant (*harvested seed number*) was estimated as:

$$\text{harvested seed number} = NNS \times TNS \times \frac{1}{2}$$

where *NSS* is the number of seeds found in two siliques and *TNS* is the total number of siliques on a given plant. Patch size and disturbance regime did not affect harvested seed number although the significance of disturbance regime was marginal (disturbance regime: $F_{1,20} = 4.087$, $p = 0.0568$; patch size: $F_{1,20} = 0.679$, $p = 0.419$, Table 7). The mean seed number in static landscapes was 1640 seeds (CI 95 %: 1124 – 2156) and it was 1940 seeds

(CI 95 %: 1727 – 2154) in dynamic landscapes. The higher seed number probably reflects lower seed mass in dynamic landscapes (also only marginally significant). Although the variance was high (Figure 8), it appears that there was no selection on seed number compared to the original lines (mean: 1980 seeds, CI 95%: 1672 – 2287) which is strange considering the selection for small seeds. The variation in all groups was also similar. Both static (Figure 8b) and dynamic (Figure 8c) landscapes showed a range similar to the original lines (Figure 8a).

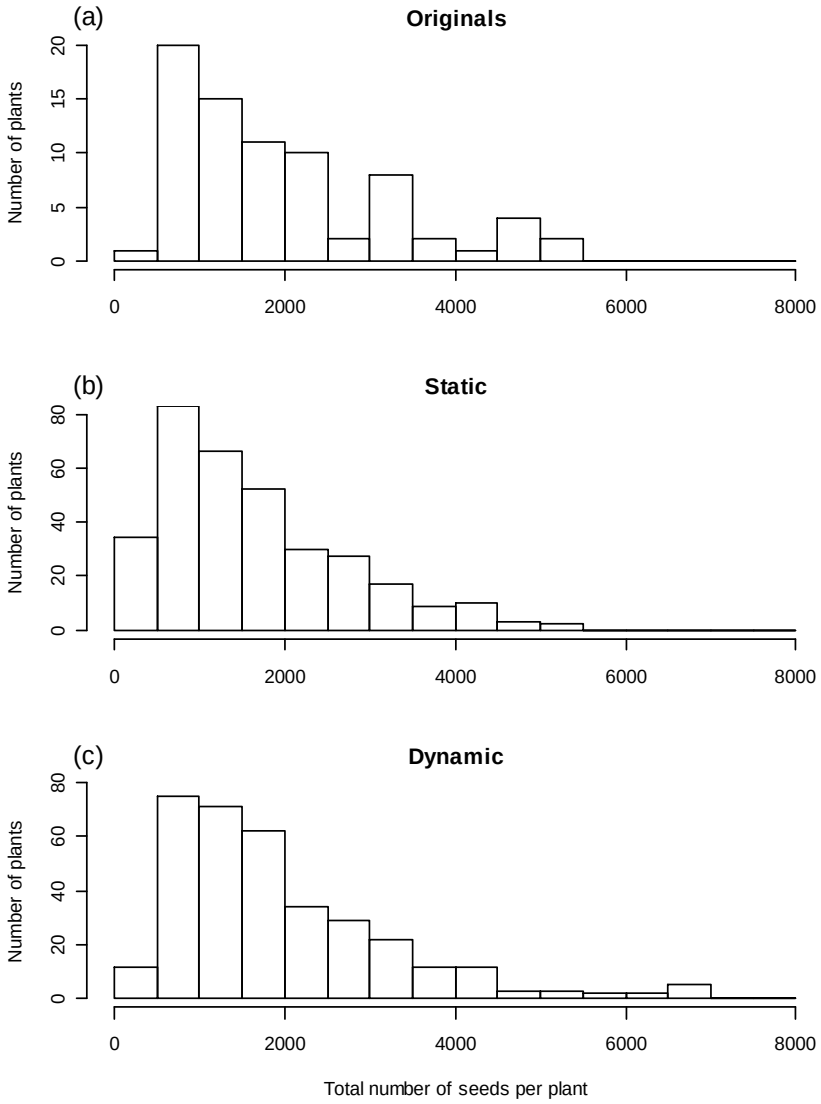

Figure 8. Histograms of total number of seeds per plant for the original lines (a), and the two disturbance regimes: static landscapes (b) and dynamic landscapes (c). For the original lines there are four individuals from each of the 19 lines. In static and dynamic landscapes there are 30 individuals from each of the 12 landscapes respectively.

Table 7. Anova table from the linear mixed-effects model of harvested seed number with the experimental treatments disturbance regime and patch size fitted as fixed effects. Landscape was treated as a random effect.

	numDF	denDF	F-value	p-value
Intercept	1	653	581	<.0001
Disturbance regime	1	20	4.087	0.0568
Log(patch.size)	1	20	0.679	0.419
Disturbance regime : log(patch.size)	1	20	0.0226	0.882

Comparison of variability

The linear mixed-effects models (*lme*) allow us to calculate the variance within and among landscapes from the random effects. In all analyses, the variance was much smaller among than within landscapes (Table 8). This indicates that the greater variation in the measured traits observed within static landscapes was probably not a result of greater variation among landscapes due, for example, to founder effects or genetic drift.

Table 8. Square root of the variance component among and within landscapes. Values taken from the random effects of linear mixed effect models. For each response variable there is more variation within than among landscapes.

Traits	Variance among landscapes	Variance within landscapes
Bolting age	0.556	1.30
Final height	15.0	55.1
Harvested seed mass	290	1164
Harvested seed number	0.346	0.614

To check that static landscapes contained more traits variation within landscapes (rather than among) we also calculated the coefficients of variation for all traits within each of the 24 landscapes (Figure 9). The means of the coefficients of variation were significantly different between the static and dynamic landscapes for the three traits: final height ($F_{1,22} = 8.8713$; $p = 0.0069$, Figure 9a), harvested seed mass ($F_{1,22} = 17.363$, $p = 0.0004$, Figure 9b) and bolting age ($F_{1,22} = 35.137$, $p < 0.0001$, Figure 9c). The coefficient of variation for seed number between static and dynamic landscapes were not significantly different ($F_{1,22} = 0.2493$, $p = 0.6225$, Figure 9d). The absolute values for the coefficient of variation for seed number are

large indicating that it is a highly plastic trait (Chapter 1). The other traits show much smaller variation, perhaps because they are under stronger genetic control (as shown for seed mass in Chapter 1).

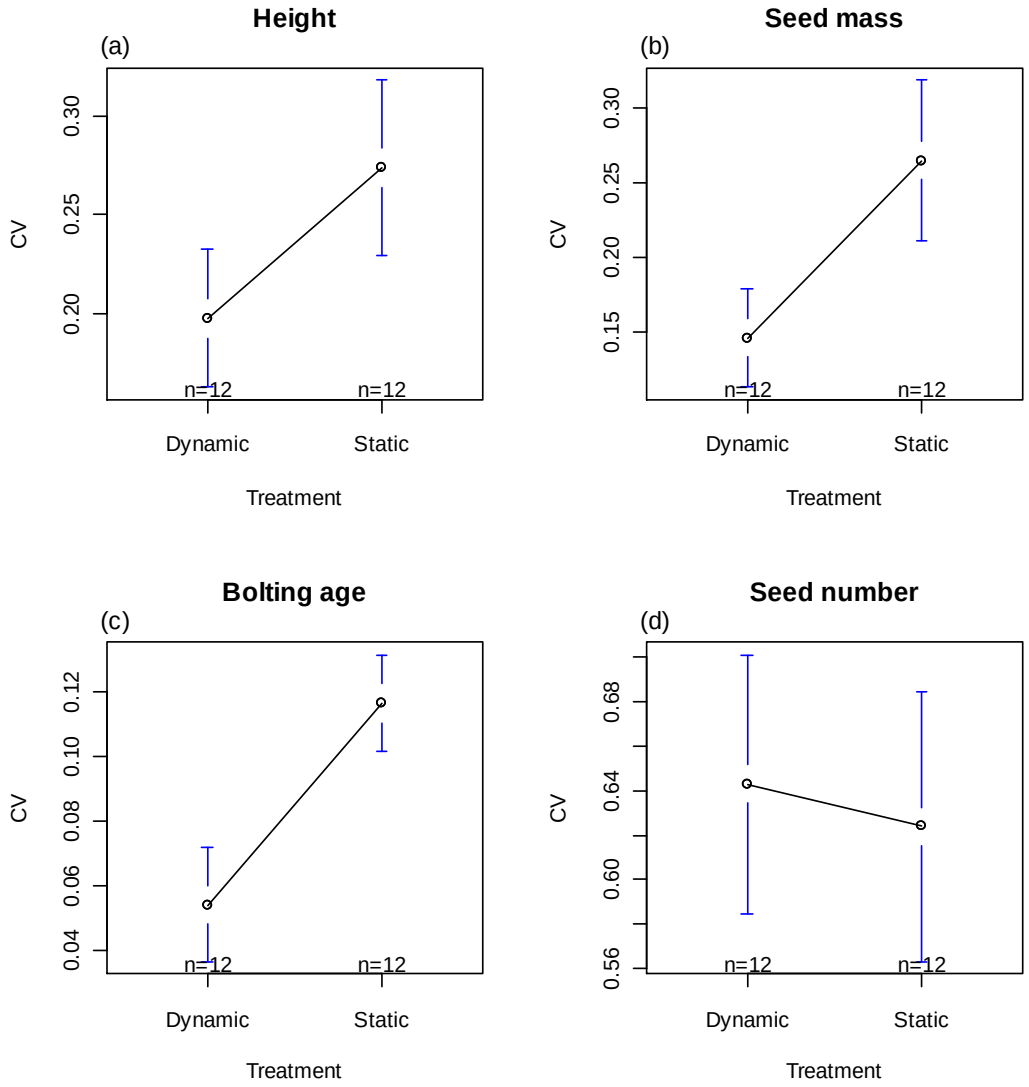

Figure 9. Relationship between coefficients of variation (CV) of final height (a), harvested seed mass (b), bolting age (c) and harvested seed number (d) in both static and dynamic landscapes.

Visualisation

In order to visualise the trait space occupied by the original lines before selection and compare this with the trait space occupied by the landscapes following selection, we generated a series of bivariate plots. We first plotted harvested seed mass, bolting age and harvested seed number in relation to final height for the 19 original lines (Figure 10). These

measurements were made on four individuals of each original line grown alongside individuals from the landscapes. Individuals from the same line normally were quite consistent and appear close together on the plots (Figure 10a, b and c). An outlier group of few lines made up of nine tall plants (> 350 mm) are also highlighted. They represent 11.84 % of the original individuals and consist of individuals belonging to only three lines.

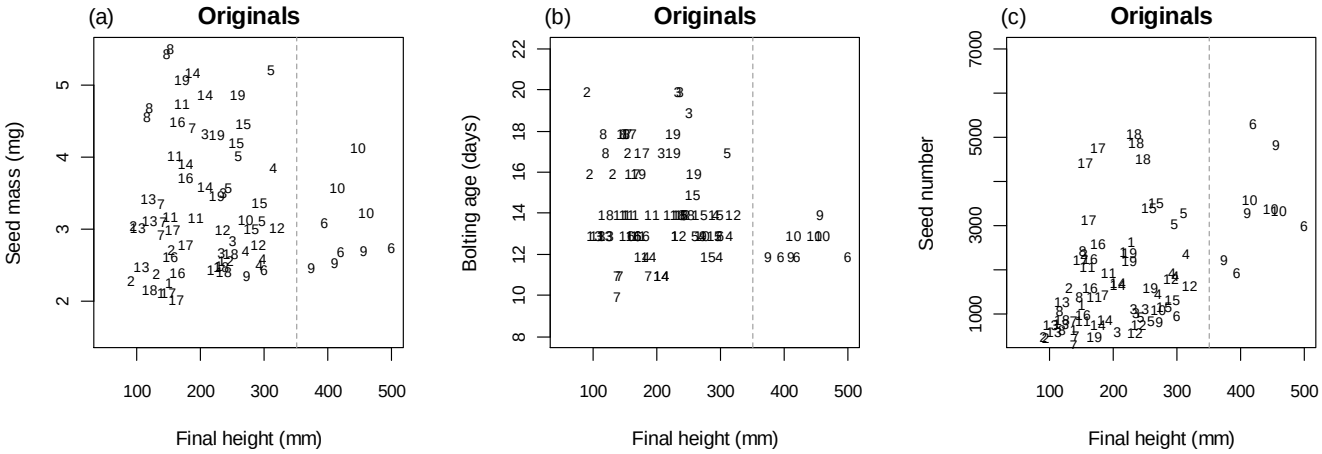

Figure 10. Relationships between harvested seed mass and final plant height (a), bolting age and final plant height (b), and harvested seed number and final plant height (c) for the original lines. Each number represents an individual from one of the original lines, which appears four times for the four replicates (for correspondence with Alonso-Blanco et al. (1999) line names see Table 1). The grey dotted-line represents an arbitrary limit to point out the outlier group of tall plants. This group of nine individuals is constituted of only three genotypes (n° 6, 9 and 10).

We then plotted harvested seed mass, bolting age and harvested seed number in relation to the final height for all individuals from the two disturbance regimes (static vs. dynamic, Figure 11). For final height vs. harvested seed mass, the original lines are evenly spread across the plot along both axes (Figure and 11a) compared to the two disturbance regimes (Figures 11b and 11c). In the original plot, there is a small group of very tall individuals which consists of nine individuals from only three genotypes (lines 6, 9 and 10). These nine individuals are above the limit of 35 cm (vertical grey dotted line, Figures 10a and 11a).

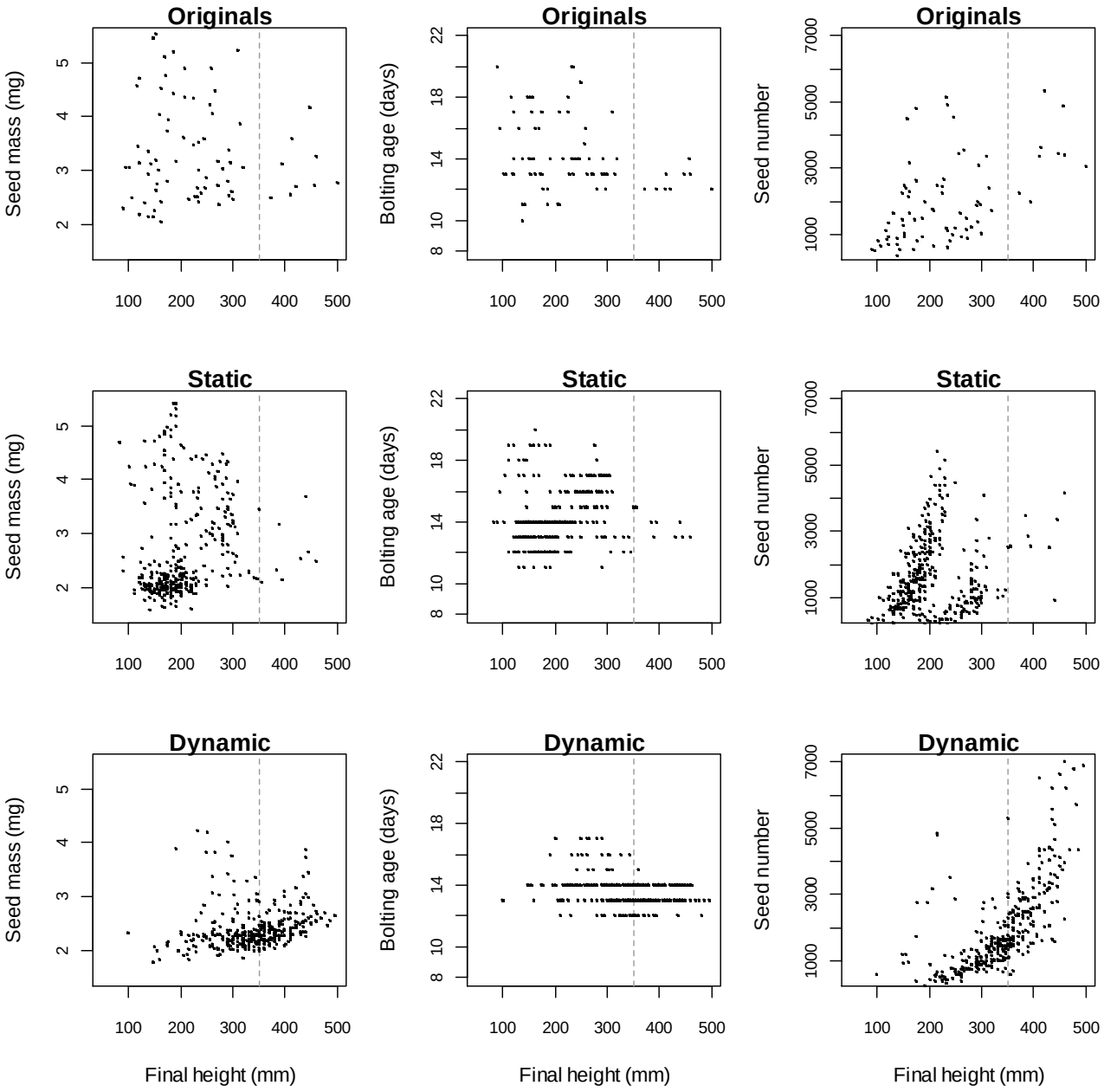

Figure 11. Relationships between harvested seed mass and final plant height (a, b, c), bolting age and final plant height (d, e, f), and harvested seed number and final plant height (g, h, i) in both disturbance regimes and for the original lines. The grey dotted-line represents an arbitrary limit to point out the outlier group of lines made of tall plants in the original lines. This group of nine individuals consists of only three genotypes (n° 6, 9 and 10).

In the static landscapes, the range along both axes is similar to the original lines but there is marked clustering in the bottom left-hand corner, showing the shift towards short small-seeded individuals (Figure 11b). In contrast, in the dynamic landscapes, the range in seed masses is greatly reduced with again strong clustering in the lower part of the seed mass range (Figure 11c). Individuals are also taller, with 43.06 % > 350 mm. This compares with only

2.22 % > 350 mm in static landscapes. By assuming that measurements on individuals belonging to the same line are highly repeatable, it is possible that the majority of the individuals selected in dynamic landscapes could belong to the three original lines constituting the outlier group (lines 6, 9 and 10). Thus dynamic landscapes could have less variation in measured traits because they selected for a very small number of lines compare to the static landscapes. After five generations of selection most individuals could therefore derive from only three genotypes.

The plot of height vs. bolting age shows a similar pattern although less extreme. The original lines cover the trait space evenly, although the outlying group of tall plants all bolt between 12 and 14 days (Figure 11d). The static landscapes have a similar range in bolting age as the original lines (Figure 11e), but in the dynamic landscapes most individuals bolt between 12 and 14 days (Figure 11f). Again, this could be because a large number of individuals in dynamic landscapes after 5 generations derive from only 3 genotypes.

The plot of height vs. seed number for the original lines clearly shows two distinct groups (Figure 11g). One group is made up of small individuals while the other group is made up of tall individuals. In fact, the first group is mainly made up of lines carrying the *erecta* mutation while the second group is mainly made up of non-*erecta* lines (Figure 12). In the static landscapes, both groups are still present (Figure 11h) while in contrast, in the dynamic landscapes the *erecta* group is lost (Figure 11i).

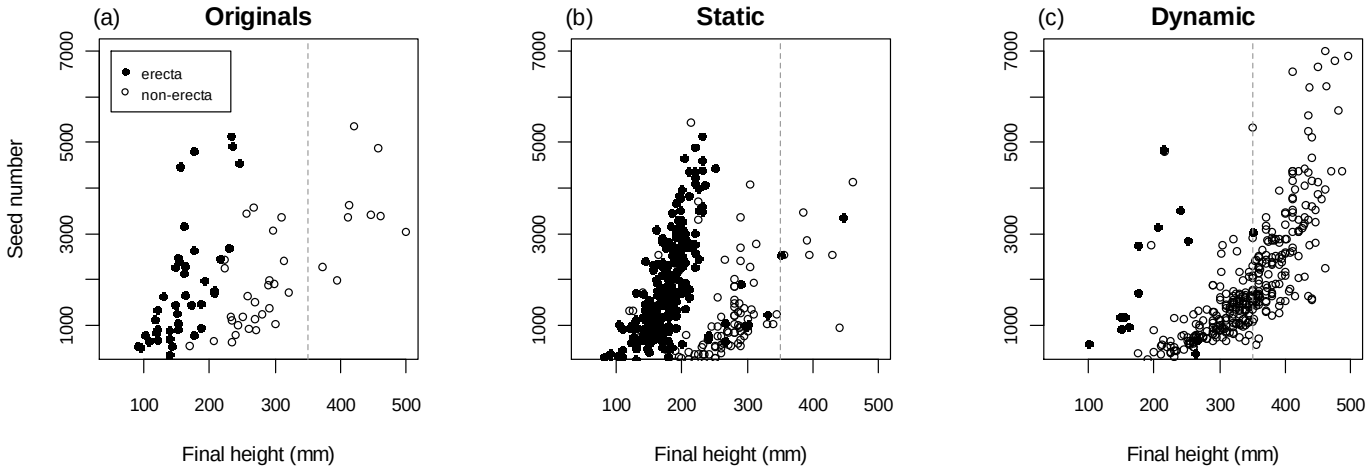

Figure 12. Relationship between seed number and final plant height in both disturbance regimes (b, c) and for the original lines (a) showing the plants carrying the *erecta* mutation (black points) and the non-*erecta* plants (white points). The grey dotted-line represents an arbitrary limit to point out the outlier group of lines made of tall plants in the original lines. This group of nine individuals consists of only three genotypes (n° 6, 9 and 10).

DISCUSSION

We grew 17 *Arabidopsis thaliana* RILs and their two parents under standardized conditions in such a way to exclude the confounding effects of density and to measure the effects of 5 generations of selection on plant traits. There was a large difference in the mean between the disturbance regimes only for final plant height. Harvested seed mass, bolting age, flowering age and seed production all experienced similar selection pressures in static and dynamic landscapes, early flowering and small seeds being favoured. Although the mean trait values were not different in static and dynamic landscapes, much less variation remained in the static landscapes indicating stronger directional selection in this disturbance regime. The reduced variation in dynamic landscapes could not only be explained by the loss of *erecta* mutation from dynamic landscapes.

We expected to find different strategies for the two disturbance regimes. The dynamic landscapes being highly disturbed habitats, a good dispersal strategy i.e. taller plants, small seeds and early flowering was expected. Small seed mass is often associated with higher dispersal because small-seeded plants tend to produce more seeds (Smith and Fretwell 1974, Turnbull et al. 1999, Nathan et al. 2002, Coomes and Grubb 2003, Wender et al. 2005) and therefore have a higher chance to colonize new patches. In addition, taller plants should disperse further. Indeed, we observed such selection in the dispersal traits for dynamic landscapes. In contrast, we expected to find a good competitive strategy for the static landscapes i.e. smaller plants, large seeds and late flowering individuals. Large seed size should confer a competitive advantage under high density conditions (Weiner and Thomas 1986, Weiner 1990, Weiner et al. 2001, Stoll et al. 2002) because they have the advantage of higher seedling survival, growth or germination success (Venable 1992, Coomes and Grubb 2003). Flowering late could also be a competitive strategy to increase shading of neighbour or take up more resources. In static landscapes, we found shorter plants as expected, but we observed selection for smaller seeds and a tendency favouring early flowering individuals. This is despite higher densities and poorer survival in static landscapes (Fakheran 2009). Contrary to what we expected, large seed mass seemed to confer no advantage. Although density was higher in static landscapes, there was no shift of the mean towards larger seeds. This is surprising because it means that large seeds do not appear to confer a size-asymmetric competitive advantage (Weiner and Thomas 1986, Weiner 1990, Weiner et al. 2001, Stoll et al. 2002). However the large-seeded lines persisted in static landscapes in comparison to the dynamic landscapes where large-seeded lines were almost eliminated. This could be explained by two possibilities. The first possibility would be that large-seeded individuals take longer to eliminate from static landscapes because the strength of selection is weaker. We would simply need to perform more generations to see them vanish. The second possibility would be that on average the density was not large enough in static landscapes to select for

large seeds, but if there was variation among patches in density, then large seeds might have an advantage in patches where the density was high enough. Maybe they would then persist over additional generations thanks to these small areas where density is very high. It also seems unlikely that seed size in static landscapes was selectively neutral (Turnbull et al. 2008)chapter) as there was an observed shift in the mean towards smaller seeds.

There was also little evidence of a difference in selection pressure for bolting/flowering time between the two disturbance regimes although the late flowering lines persisted better in the static landscapes. This could be due to the artificial generation time imposed. This generation time was actually the same between static and dynamic landscapes. However, we did observe the persistence of some late flowering individuals in static landscapes which were eliminated from dynamic landscapes. It is unclear whether this is due to weaker directional selection or genuine disruptive selection which preserves both early and late flowering genotypes.

Finally the landscapes variance was much smaller among than within landscapes (Table 8). This indicates that the greater variation in the measured traits observed within static landscapes was probably not a result of greater variation among landscapes due, for example, to *founder effects* or *genetic drift*. The *founder effect* contributes to *genetic drift*, which causes certain genetic traits to vanish or become more abundant (Ridley 2004). In the static landscapes, traits becoming more abundant or vanishing were not observed. The greater variation within static landscapes might therefore be explained by other processes.

In dynamic landscapes a there was clearly strong selection for taller plants. As shown in Fakheran (2009) there is a negative correlation between seed mass and height for the original lines. Therefore a strong selection on plant height leads inevitably to selection on small seeds. Only few individuals were very tall and they were small-seeded (Genotypes n°6 and 9 from the original lines, Figure 10a). There is some ability to select height and seed size independently with a genotype which exhibit quite a large variation in seed mass and which is quite large-seeded (Genotype n°10 from the original lines, Figure 10a). If selection operated

mainly on seed size in dynamic landscapes, small-seeded individuals carrying the *erecta* mutation should have been equally fit, but they were almost eliminated from the dynamic landscapes. Thus selection was probably primarily on height and not seed size.

It was claimed that diversity declines in more productive environments due to increased impact of competitive exclusion. This relationship was acknowledged by numerous studies (Grime 1973, 1979, Begon et al. 1996, Grace 1999, Keddy 2005). Disturbance had also been questioned in its role of promoting species coexistence (Chesson and Huntly 1997, Zobel and Partel 2008). The negative effects of both competition and disturbance led to the concept of intermediate-disturbance hypothesis (IDH). IDH is one of the most frequently suggested explanations for the maintenance of species diversity in ecological communities (Connell 1978, Wilson 1990, Roxburgh et al. 2004). The intermediate level of disturbance prevents competitive exclusion by dominant species or genotypes, and hence maximise diversity. However, our results showed that the static landscapes had a larger variance for several traits as seed mass and height compare to the dynamic landscapes although they both experienced a similar selection pressure. Disturbance seemed to have imposed a much stronger selection pressure than competition. A rapid elimination of many original lines seemed to have occurred in dynamic landscapes, while in static landscapes a greater variety of lines survived despite the higher levels of seedling death. Our results show that a greater variety of genotypes survived in static landscapes. It is rather unexpected and indicates that disturbance may be a more potent force for genetic variation decline than competition in annual plants.

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CHAPTER 5

Growth rates, seed size, and physiology:

Do small-seeded species really grow faster?

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GROWTH RATES, SEED SIZE, AND PHYSIOLOGY: DO SMALL-SEEDED SPECIES REALLY GROW FASTER?

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Abstract. Relative growth rate (RGR) is currently the most commonly used method for measuring and comparing species' intrinsic growth potential. Comparative studies have, for example, revealed that small-seeded species have higher RGR, leading to the common belief that small-seeded species possess physiological adaptations for rapid growth that would allow them to outgrow large-seeded species, given sufficient time. We show that, because RGR declines as individual plants grow, it is heavily biased by initial size and does not measure the size-corrected growth potential that determines the outcome of competition in the long term. We develop a daily growth model that includes a simple mechanistic representation of aboveground and belowground growth and its dependency on plant size and environmental factors. Intrinsic growth potential is encapsulated by the size-independent growth coefficient, *G*. We parameterized the model using repeated-harvest data from 1724 plants of nine species growing in contrasting nutrient and temperature regimes. Using information-theoretic criteria, we found evidence for interspecific differences in only three of nine model parameters: *G*, aboveground allocation, and frost damage. With other parameters shared between species, the model accurately reproduced above- and belowground biomass trajectories for all nine species in each set of environmental conditions. In contrast to conventional wisdom, the relationship between *G* and seed size was positive, despite a strong negative correlation between seed size and average RGR, meaning that large-seeded rather than small-seeded species have higher size-corrected growth potential. Further, we found a significant positive correlation between *G* and frost damage that, according to simulations, causes rank reversals in final biomass under daily temperature changes of ±5°C. We recommend the wider use of this new kind of plant growth analysis as a better way of understanding underlying differences in species' physiology; but we recognize that RGR is still a useful metric if considering the potential rate of population increase in empty habitats.

Key words: coexistence; community ecology; ecophysiology; European sand-dune annual species; exponential vs. linear growth; likelihood; neutral theory; relative growth rate (RGR); storage effect; trade-offs.

Introduction

The variation in seed size within functionally similar guilds is higher than almost any other measurable feature of coexisting plants (Salisbury 1974, Lord et al. 1995, Moles et al. 2005). One possible explanation for this variation is that species evolve different seed sizes under a competition–colonization trade-off and that small-seeded species are therefore better colonizers but are not physiologically distinct (Tilman 1994, Geritz 1995, Rees and Westoby 1997, Geritz et al. 1999, Jakobsson and Eriksson 2000, Levine and Rees 2002, Coomes and Grubb 2003, Turnbull et al. 2004). A second, related possibility, is that small seeds are one of a suite of adaptations to a spatial successional or pioneer niche (Grime 1979, Tilman 1982, Pacala and Rees 1998, Bolker and Pacala 1999), in which case small-seeded species should possess additional physiological adaptations for rapid growth (Tilman 1982, Pacala et al. 1996, Davies 2001). The well-documented negative interspecific correlation between seed size and relative growth rate (RGR; Gross 1984, Maranon and Grubb 1993, Reich et al. 1998, Poorter and Rose 2005) seems to support the idea that small-seeded species are inherently faster growing. But conventional measures of RGR contain an intrinsic size bias.

The RGR problem

The evidence that small-seeded species have higher RGR comes mainly from pot experiments in which different species are grown under standardized conditions and average RGR is calculated over the entire growth period (e.g., Gross 1984, Maranon and Grubb 1993). However, it is well-documented that the instantaneous RGR expressed by an individual plant usually declines as it grows (Grime and Hunt 1975, Hunt 1982, Enquist et al. 1999). Whatever the cause, this decline in

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RGR means that, all else being equal, plants that start growth at smaller sizes—e.g., small-seeded plants—should exhibit a higher average RGR over any subsequent period. Even different-sized individuals of a single species, which are expected to have identical instantaneous growth rates at a given size (Fig. 1A), when grown in pots and harvested after some fixed time interval would have different values of average RGR. The size dependency of RGR means that average RGR measured in the usual way is, at least partly, an artefact of initial size (Fig. 1). If the size-dependency of RGR is large, the true relationship between seed size and growth physiology could be masked, and important trade-offs obscured, e.g., between growth rates in high vs. low light levels (Kitajima and Bolker 2003, Sack and Grubb 2003).

Size- and environment-dependent growth

One possible remedy to this situation is to conduct experiments with multiple harvests and to fit standardized time-dependent growth curves, such as the logistic or Gompertz, that implicitly assume declining RGR (Hunt 1982). Instantaneous growth rates for some standard size could then be calculated and compared between species (Metcalf et al. 2006). However, such techniques have other disadvantages. First, the parameters of the curves usually have no clear biological meaning (e.g., the inflection point of the logistic curve). Second, the technique could only work in a perfectly constant environment (such as might be created in a growth cabinet); otherwise, species that begin growth at different sizes reach any given size at different times, with different environmental conditions (Egli and Schmid 2001). Thus size and environmental effects become confounded. Third, it is not clear how to extend the use of standard growth curves to include different plant compartments (e.g., shoots and roots) that are fundamentally linked by shared processes (e.g., carbon fixation, allocation).

A mechanistic approach

In this paper we use an entirely different approach where, instead of fitting a time-dependent growth curve, we develop a mechanistic growth model that predicts the daily change in size given the conditions on that day. We believe that a mechanistic model has several advantages: alternative formulations for the size–growth relationship can be readily compared; above- and belowground growth can be modeled simultaneously, via allocation of carbon to above- vs. belowground tissue; physiologically reasonable relationships between environmental conditions (in this case, temperature and day length) and carbon fixation can be specified; and periods of tissue loss, such as that induced by frost damage, can be incorporated easily. Models formulated in this way can capture the fact that plant growth often bears little relationship to the idealized time-dependent forms specified by growth curves, showing instead irregular growth rates and periods of loss. In addition, the parameters of a mechanistic model have clear biological meanings (e.g., growth–temperature optima, fractional allocation to belowground parts) and this makes model interpretation much simpler. Once parameterized, it is easy within a mechanistic framework to perform further simulations of growth under alternative scenarios (e.g., increased daily temperatures). And most important for our purposes, the model allows unbiased comparison of the physiology of different species through the estimated size-independent parameters. In this case, we specify a size-independent growth coefficient, G.

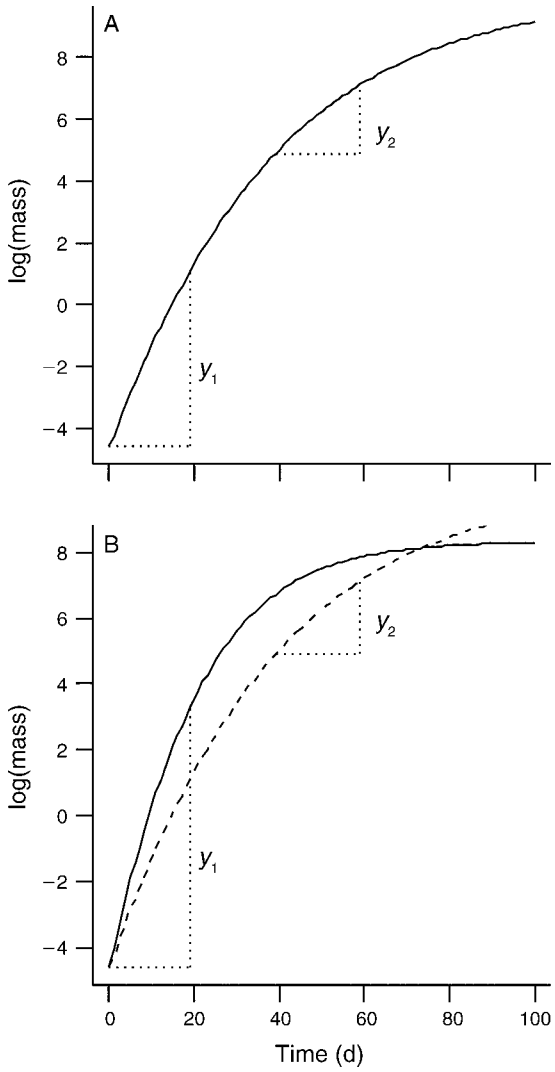

FIG. 1. If instantaneous RGR (relative growth rate) declines with size, species that begin growth at a smaller mass will always have higher average RGR ($y_1 > y_2$) whether (A) two species have exactly the same instantaneous growth rate at a given size (in this case a Gompertz function) or (B) small-seeded species actually grow faster for a given size and can therefore outgrow the larger-seeded species at least initially. A negative correlation between RGR and initial size cannot therefore distinguish between these two alternatives.

We parameterize the model using over 9000 repeated measures of above- and belowground biomass (both destructive and nondestructive) from an experiment involving nine species of sand-dune annuals grown in a variety of nutrient and temperature regimes. Our analysis reveals, among other things, that the observed negative relationship between seed size and RGR is entirely due to the common growth–size relationship that species share and that large-seeded species generally have higher size-independent growth potential.

Methods

The growth experiment

We grew 1724 individuals of nine common European sand-dune annual species from seed. Although competitive interactions between these species have been intensively studied (e.g., Mack and Harper 1977, Rees et al. 1996, Coomes et al. 2002, Turnbull et al. 2004), little is currently known about their specific growth characteristics. Plants were grown in individual cells and watered regularly with one of five different dilutions of a complete nutrient solution ($N = 0$, 0.25, 0.5, 0.75, 1; see Plate 1). Above- and belowground parts were regularly harvested from September 2003 to April 2004 (a total of seven harvests). All plants were initially outside in an experimental garden; however, after five weeks, half of the plants were brought inside to a cool greenhouse where they were protected from frost damage. Daily temperature records were obtained for plants both inside and outside. Hours of daylight on each day of the experiment were calculated using the formula presented in Forsythe et al. (1995). From harvest number 4 onwards, we also took nondestructive measures (height and diameter) of all harvested plants. Using the resulting regression model between the destructive and nondestructive measures, we then predicted the biomass of unharvested plants from which the same nondestructive measures were taken; although these data were treated differently from those collected directly from destructive sampling (for more details on species and growing conditions see Appendix A).

Daily growth model

The daily growth model is intended as a simple mechanistic representation of the growth process and its dependency on plant size and environmental factors. The model had to be kept to a level of simplicity appropriate to the data; thus, individual physiological processes are treated phenomenologically (e.g., the use of net whole-plant daily carbon gain), simple functional forms are assumed (e.g., growth vs. temperature), and some complexities are ignored (e.g., ontogenetic shifts to reproduction). Nonetheless, in comparison to traditional statistical methods, this approach allows an increased level of understanding of the physiological differences underpinning whole-plant patterns of growth, with little or no increase in the number of required parameters.

For a given individual plant i and time d (days after the start of the experiment), the daily growth model calculates a daily mass increment both aboveground ($\Delta M_{i,d}^{(\mathrm{abv})}$) and belowground ($\Delta M_{i,d}^{(\mathrm{blw})}$):

$$\Delta M_{i,d}^{(\mathrm{abv})} = F_{i,d} \times C_{i,d} - \mu_c \times H_d M_{i,d}^{(\mathrm{abv})} \quad (1a)$$

$$\Delta M_{i,d}^{(\mathrm{blw})} = (1 - F_{i,d}) C_{i,d} \quad (1b)$$

where $C_{i,d}$ is net daily carbon gain (mg) (see Eq. 2, below), and $F_{i,d}$ is the fraction of this gain that is allocated to aboveground tissue (notice that the total growth increment on any day when $T_d \geq 0$ is simply $C_{i,d}$). The parameter μ_c is a fractional loss of aboveground tissue (d^{-1}) that occurs when and only when the mean daily temperature (T_d) is below zero ($H_d = 0$ when $T_d \geq 0$ and $H_d = 1$ when $T_d < 0$).

Carbon gain vs. size.—In order to determine the daily growth increment we first needed to specify the underlying relationship between growth (i.e., the carbon gain $C_{i,d}$), and size. Nearly all commonly used plant-growth functions approximate the canonical sigmoid growth curve, which has an initial phase where growth is close to exponential, followed by a second phase where growth is close to linear, followed by a third phase in which growth declines to zero. In the initial phase growth is proportional to mass (giving constant relative growth rate [RGR] and hence increasing absolute growth rate). In the second phase growth is more or less independent of mass (giving constant absolute growth rate and hence declining RGR). We built these two phases into our daily growth model in the simplest way possible, by assuming that growth switches abruptly from an initial phase, where carbon fixation is proportional to aboveground biomass, to a second phase, where carbon gain is independent of aboveground biomass (the third phase, representing senescence, is ignored). The switch occurs when the aboveground mass reaches a critical mass, M_{ref}, which is a parameter of the model:

$$C_{i,d} = \begin{cases} G \times B_d \times \left(M_{i,d}^{(\mathrm{abv})} / M_{\mathrm{ref}} \right) & \text{if } M_{\mathrm{abv}} < M_{\mathrm{ref}} \\ G \times B_d & \text{if } M_{\mathrm{abv}} \geq M_{\mathrm{ref}} \end{cases} \quad (2)$$

where G is the size-independent growth coefficient and B_d is a multiplier that adjusts growth according to nutrients and to the temperature and day length on day d. These assumptions mean that, in a constant environment and with constant allocation to aboveground tissue (see *Allocation*, below), plants will grow exponentially until they reach aboveground biomass M_{ref}, and then switch to linear growth. But unlike standard growth curves, the daily growth model can be implemented in a varying environment with size- and resource-dependent allocation. Within the model-fitting process, M_{ref} is free to take any positive value. Therefore, if plants grow either linearly for the entire time or exponentially for the entire time, the best value

of M_{ref} will be, respectively, so small or so large that no plant actually exhibits such a switch.

This formulation for the relationship between size and growth was the best that we could find to fit to our data, after extensive consideration of alternatives (including power functions of net carbon gain vs. size) and is also particularly simple to analyze and understand. We also experimented with formulations explicitly separating carbon fixation and respiration but found that the parameters were underconstrained given the nature of the data. However, other formulations could easily be used within this general model framework.

Nutrients, temperature, and day length.—The coefficient B_d in Eq. 2 allows the incorporation of environmental conditions—in this case the nutrient level (N), mean daily temperature T_d, and day length L_d:

$$B_d = \exp(\alpha N) \times (1 - H_d) \times \exp\left[-\left(\frac{T_d - T_{\text{opt}}}{\sigma_t}\right)\right] \times \left(\frac{L_d}{12}\right). \quad (3)$$

The growth–nutrient response is a simple exponential function of nutrient concentration whose steepness is determined by the parameter α, where $\alpha > 0$ indicates a positive response to nutrients. The growth–temperature response is a Gaussian function, which reaches a value 1 when T_d is equal to an optimum temperature, set by the parameter T_{opt}, and which shows a symmetric decay either side of T_{opt} with a steepness set by the parameter σ_t (smaller σ_t giving a steeper response). This function was chosen because it provided a superior fit compared to nonsymmetric functions. Eq. 3 includes the additional assumption that carbon gain is zero when $T_d < 0$ (note the use of H_d). Any differences in the growth response of species to nutrient availability and temperature would be reflected in species-specific values for these parameters (α, T_{opt}, and σ_t). Note, that any differences in plant growth in the two locations (inside and outside) are assumed to be solely due to differences in average daily temperatures T_d and the occurrence (outside only) of sub-zero temperatures.

Consideration of Eqs. 2 and 3 reveals that G, the size-independent growth coefficient, affects growth at all sizes and under all nutrient levels. More precisely, G is the maximum absolute growth rate under zero nutrients, i.e., the growth increment per day achieved when $M_{i,d}^{(\text{abv})} \geq M_{\text{ref}}$, $T_d = T_{\text{opt}}$, and $N = 0$.

Allocation.—The fractional aboveground allocation $F_{i,d}$ is given by

$$F_{i,d} = 1/[1 + \exp(-A_{i,d})] \quad (4a)$$

$$A_{i,d} = \gamma_0 + \gamma_{\text{M}}\left[M_{i,d}^{(\text{abv})} + M_{i,d}^{(\text{blw})}\right] + \gamma_{\text{N}} N. \quad (4b)$$

Here, Eq. 4a is a logit function, bounding $F_{i,d}$ between 0 and 1 ($F_{i,d} = 0.5$ when $A_{i,d} = 0$), and $A_{i,d}$ is a linear function, including the parameter γ_0 as a constant, and the parameters γ_{M} and γ_{N} to set, respectively, the effects of biomass and nutrients on allocation.

Parameter estimation

The model required the estimation of nine parameters: G, M_{ref}, γ_0, γ_{M}, γ_{N}, T_{opt}, σ_t, α, and μ_{c}. We used maximum-likelihood methods to estimate global values for these parameters or for each species separately, given the data from the growth experiment (for detailed description see Appendix B). An important aim of the analysis was to estimate which aspects of the physiology differ between species, i.e., which of the nine model parameters are species specific, and which are global (shared between species). This was achieved by comparing information criteria from model fits where different combinations of the nine parameters were made species specific or global. The set of all such combinations was too large ($2^9 = 512$ models), so we began by fitting a model with all nine parameters global, and then fit nine models with each parameter in turn made species specific. From this set of nine models, we selected the model with the greatest likelihood and set the relevant parameter (p_1) to be permanently species specific. We then fit all eight models with two species-specific parameters, one of which was always p_1. From these eight, the model with the greatest likelihood was chosen, thus fixing p_2, and so on until all nine parameters had been made species specific. This required 46 model fits in total. Comparing the AIC (Akaike information criterion) and BIC (Schwarz/Bayesian information criterion) of this set of 46 models allowed us to decide on the most appropriate models from the 46 (see *Results: Model selection*, below, and Burnham and Anderson 2002). In addition, the order in which this procedure sets a given parameter to species specific indicates the extent to which the data and model structure imply that this parameter is species specific: p_1 is the parameter with the strongest evidence, and p_9 is the parameter with the least.

Model–data comparison

After parameterization, we implemented simulations of the *global model* (where all parameters are shared between species) and the model selected using BIC (which had three species-specific parameters, referred to as the "3-p model," see *Results: Model selection*, below), for each of the different nutrient and temperature regimes used in the growth experiment. We calculated, using the predicted biomasses from the global and 3-p models: (1) a predicted average RGR in each nutrient level and temperature regime. In addition, by performing linear regressions of predicted final aboveground biomass against log nutrient concentration for each species both inside and outside, we calculated: (2) a predicted relative response of final biomass to temperature regime, defined as the ratio of the intercepts (inside vs. outside) and (3) a predicted relative response of final biomass to nutrient addition, both inside and outside,

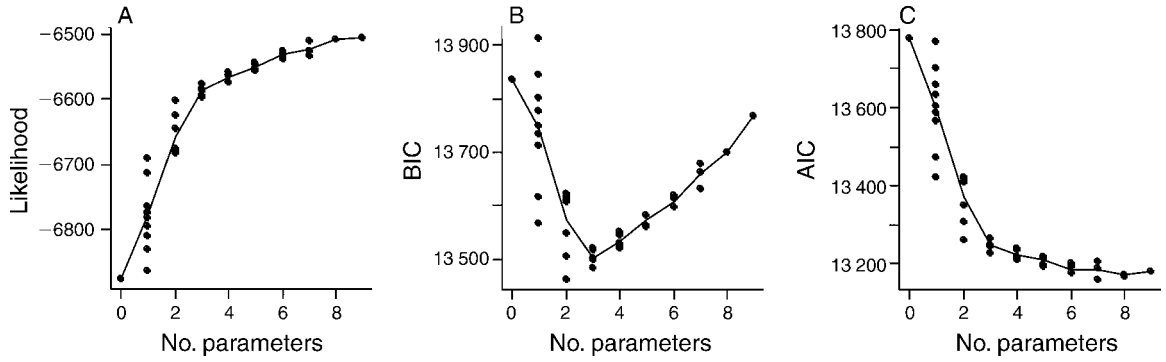

FIG. 2. Comparison of (A) likelihood, (B) Schwarz/Bayesian information criterion, BIC, and (C) Akaike information criterion, AIC, associated with each of the 46 fitted models and the number of species-specific parameters that each model contains. There is only one model with no species-specific parameters, nine with one species-specific parameter, eight with two, and so on. The trend lines connect averages for models with the same number of species-specific parameters.

defined as the ratio of predicted final biomass in full-strength vs. zero-strength nutrients. For comparison, the same metrics were calculated using the observed data.

RESULTS

RGR and response to nutrients and temperature

There was a near-perfect rank correlation between seed size and initial mass after 14 days ($r_s = 0.967$, $n = 9$ species, $P < 0.001$). Only *Valerianella* had a substantially lower biomass after 14 days than expected from its seed size. Analysis of average RGR (relative growth rate) from week 2 to week 19 revealed the expected strong negative relationship between RGR and seed size ($F_{1,46} = 639$, $P << 0.0001$), with *Saxifraga* achieving RGRs roughly 4 times higher than *Erodium*. In addition, there was a significant interaction between seed size and nutrient treatment ($F_{1,46} = 10.5$, $P = 0.0023$) and between seed size and temperature regime ($F_{1,46} = 9.78$, $P = 0.0031$) such that the relationship between seed size and RGR was steeper in higher nutrient levels, and steeper outside than inside. Correspondingly, the final biomass of small-seeded species showed a greater relative response to nutrient addition when grown inside ($F_{1,7} = 15.2$, $P = 0.006$) but not outside, ($F_{1,7} = 1.31$, $P > 0.05$). Similarly, the final biomass of small-seeded species showed a greater relative response to increased temperatures (inside vs. outside) ($F_{1,6} = 14.87$, $P = 0.008$) once a single strongly outlying point (*Valerianella*) was removed. Thus, conventional growth analysis reveals that small-seeded species have higher RGR, and show a greater relative increase in final biomass when either nutrients (inside only) or temperatures are increased.

Model selection

Fig. 2 compares the likelihood, AIC, and BIC values from the set of 46 daily growth models considered in the model selection procedure (see *Methods: Parameter estimates*, above). Visual inspection of the likelihood suggested that it improved sharply when the number of species-specific parameters was increased from zero to three, whereas making additional parameters species specific led to rather more modest improvements (Fig. 2A). This was reflected in the BIC, which on average picked a model with three species-specific parameters (Fig. 2B). In contrast the AIC selected a model with eight species-specific parameters (Fig. 2C; hereafter the "8-p model"). Although still debated, there is at least some agreement that the AIC should be preferred when the main goal is predictive accuracy, while the BIC, which penalizes complexity much more heavily, may be preferred if the goal is to identify key important processes (Taper 2004).

Model–data comparison

Comparing the predictions from the 3-p and 8-p models (Fig. 3) showed that the improved accuracy of the 8-p model was restricted to particular species in particular situations. For example, the 8-p model performed noticeably better for *Erodium* grown outside (Fig. 3I). However, because the differences in model fit are minor, and because the improvement may come as much from structural inadequacies of the model on some occasions rather than genuine interspecific differences in physiology, we do not consider the 8-p model further. However, the global model is of particular interest because in the global model the only difference between the species is the initial mass (which is highly correlated with seed size).

Species-specific physiology

The strength of evidence for species-specific (rather than global) values of the different parameters is given by the order in which the model-selection procedure made the parameters species specific (Table 1). The parameters with the strongest such evidence were (1) the size-independent growth coefficient, G; (2) baseline allocation, γ_0; and (3) the cold-damage parameter, μ_c (hence these parameters were retained in our 3-p model). In contrast, M_{ref}, the mass at which growth switches

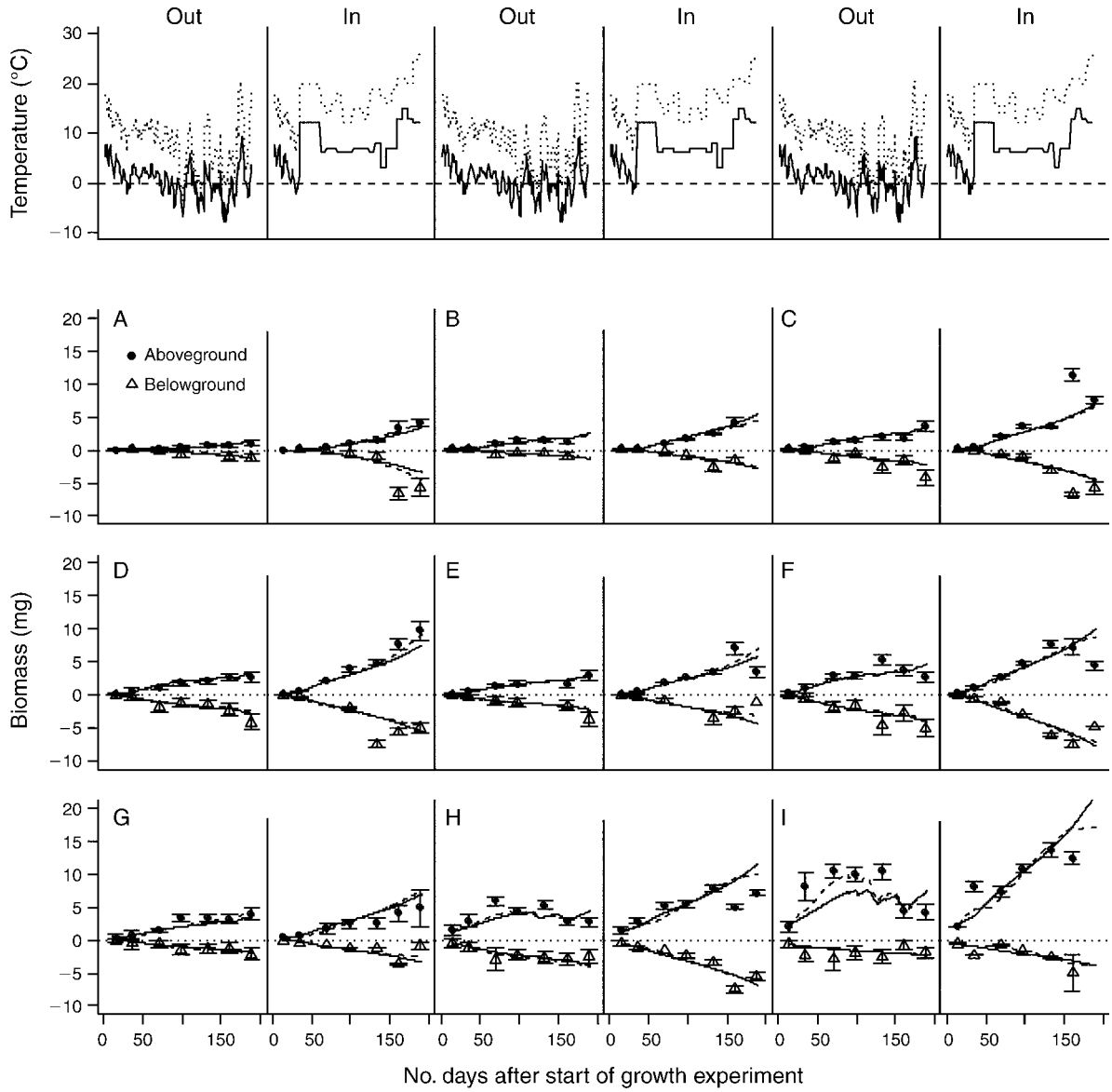

FIG. 3. Temperature and growth curves for the nine species (common European sand-dune annuals) in the growth experiment, recorded both outside in the experimental garden (Out) and inside a greenhouse with frost protection (In). (Top row) Maximum (dotted line) and minimum (solid line) temperatures were recorded. (Rows 2–4) Aboveground (solid circles, •) and belowground (open triangles, △) biomass data (geometric means ± SE) at one nutrient concentration ($N = 0.25$, one quarter full strength) are plotted, together with fitted growth curves from the best three-parameter (3-p) model (solid line) and the best eight-parameter (8-p) model (dashed line). Day 0 is the day seeds were sowed. Species are plotted in order of ascending seed size: (A) *Saxifraga*, (B) *Erophila*, (C) *Cerastium*, (D) *Arenaria*, (E) *Veronica*, (F) *Myosotis*, (G) *Valerianella*, (H) *Geranium*, and (I) *Erodium*.

from exponential to linear, was one of the last parameters to be made species specific. This is an important result, allowing us to unambiguously rank the species in terms of their growth potential, according to the size-independent parameter G.

Inspection of parameter estimates from the global model show that the fractional belowground allocation declines as nutrient availability increases ($\gamma_N > 0$), and as size increases ($\gamma_M > 0$); the optimum growth temperature is around 13°C (Table 1).

Parameters vs. seed size

The maximum-likelihood estimates of the species-specific parameters retained by the 3-p model (selected by the BIC) were all positively related to seed size (Fig. 4A–C). Thus, the analysis estimated that larger-seeded species have higher size-independent growth coefficients (greater G; $F_{1,7} = 9.08$, $P = 0.019$) and allocate less carbon to belowground tissue (greater γ_0; $F_{1,7} = 6.19$, $P = 0.041$), but are more susceptible to cold damage

TABLE 1. Description of parameters used in the growth model and the order in which species-specific parameters are retained in the stepwise model-fitting procedure.

Symbol	Definition	Units	Order retained	Global values†
G	Size-independent growth coefficient	mg/d	1	0.132
μ_c	Fractional tissue loss (when mean daily temperature $T_d < 0$)		3	0.00357
M_{ref}	Growth: critical mass at which there is switch from exponential to linear	mg	7	0.564
T_{opt}	Growth: optimum temperature	°C	6	12.6
σ_t	Growth: sensitivity to temperature (standard deviation of Gaussian response)	°C	4	10.8
α	Growth: coefficient for effect of nutrients		8	0.453
γ_0	Allocation: constant		2	0.199
γ_M	Allocation: coefficient for effect of plant mass		5	0.0524
γ_N	Allocation: coefficient for effect of nutrients		9	0.235

† Values of each parameter are given for the global model (in which no parameters are species specific).

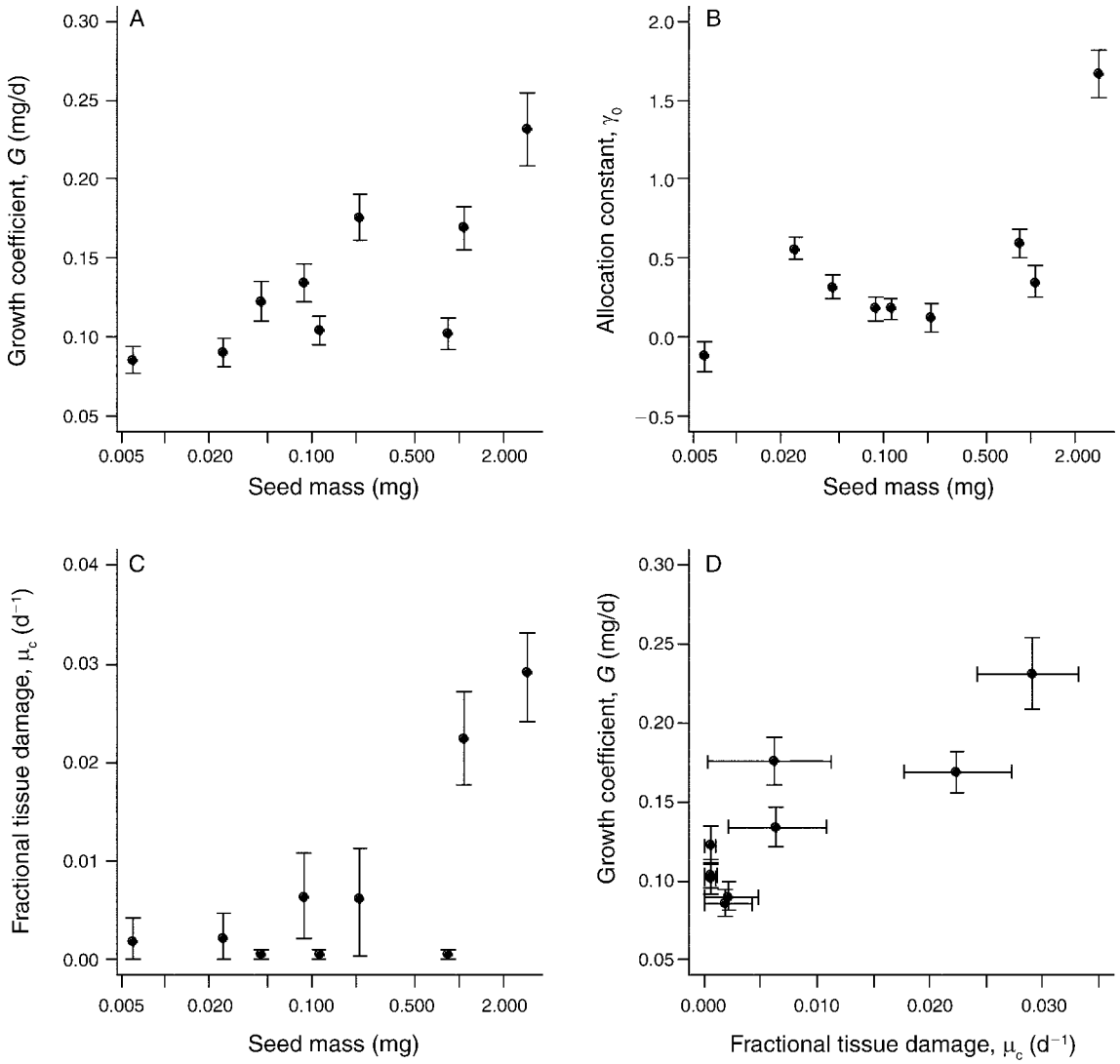

FIG. 4. The relationship between seed size (mass) and the species-specific parameters retained in the best three-parameter model for nine species: (A) size-independent growth coefficient (G), (B) baseline aboveground allocation constant (γ_0), (C) fractional loss of aboveground tissue due to cold (μ_c), and (D) the trade-off between G and μ_c. For (A)–(C) note the x-axis log scale.

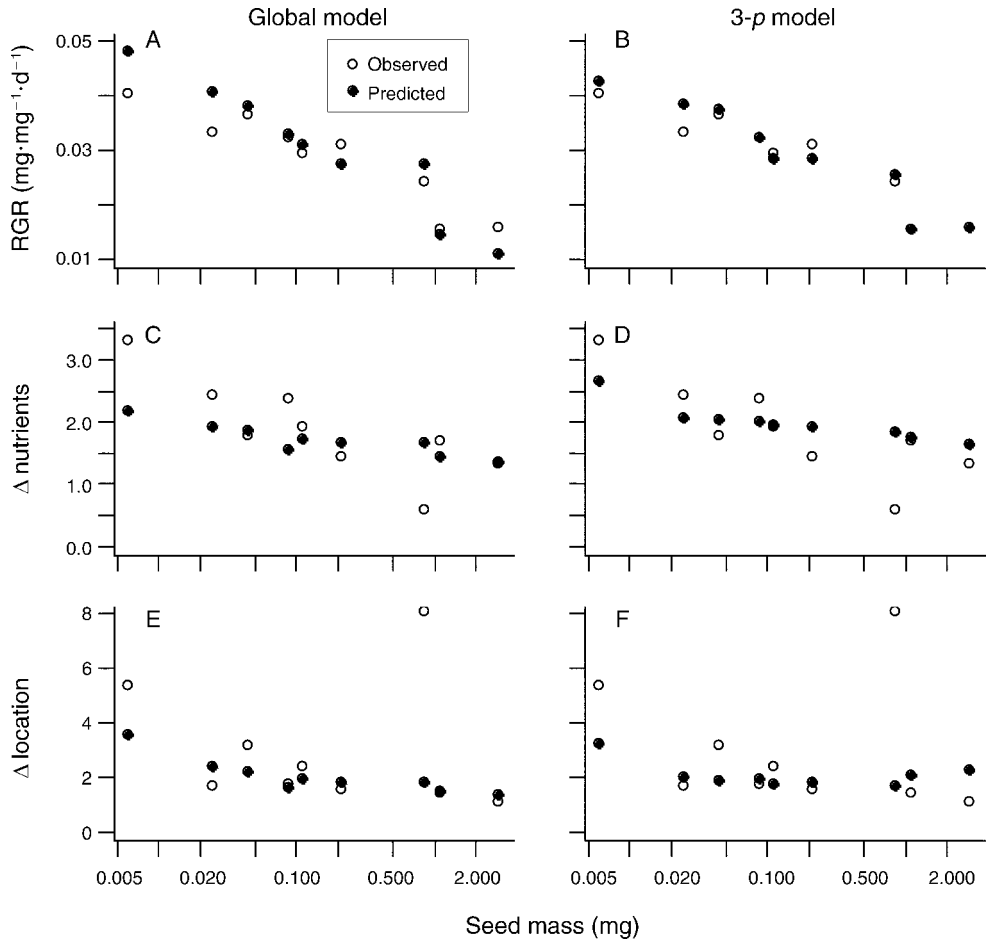

FIG. 5. Observed and predicted mean relative growth rate (RGR) and growth responses to nutrient level and location for nine species under the global model and the 3-p model, from week 2 to week 19. In the global model, all parameters are shared between species; the 3-p model (selected using BIC) has three species-specific parameters. (A, B) Mean RGR inside the greenhouse, shown at only one nutrient level ($N = 0.25$) for clarity. (C, D) Relative change in final biomass due to nutrient addition. (E, F) Relative change in final biomass due to increased temperature inside greenhouse (vs. outside). Seed mass of the nine species (x-axis) is shown on a log scale.

(greater μ_c; $F_{1,7} = 7.41$, $P = 0.030$). However, the relationship between these parameters and seed size was not perfect: in particular, *Valerianella* is large-seeded, but has a low value of G. Plotting G against μ_c revealed a significant positive correlation between these two parameters (Fig. 4D; $\rho = 0.875$, $n = 9$ species, $P = 0.002$) such that species with higher size-independent growth coefficients experience more tissue loss when temperatures fall below zero. This relationship ($r^2 = 0.77$) was better than the relationships between both G and seed size ($r^2 = 0.57$) and μ_c and seed size ($r^2 = 0.51$) suggesting that the trade-off between G and μ_c may be inescapable; high size-independent growth coefficients come at the cost of high cold damage.

Predicted response metrics vs. seed size

Analysis of simulated data from the global model up to week 19 revealed that small-seeded species are predicted to (1) have higher average RGR ($F_{1,7} = 95.8$, $P < 0.001$; Fig. 5A); (2) show a higher relative increase in their final biomass with increased temperatures ($F_{1,7} = 20.8$, $P = 0.003$; Fig. 5E), and (3) show a higher relative increase in their final biomass with additional nutrients ($F_{1,7} = 38.1$, $P < 0.001$; Fig. 5C). This was despite the fact that the global model had no species-specific parameters. With respect to points (1)–(3) above, the differences between the global and 3-p model were small (Fig. 5), suggesting that the species-specific aspects of physiology estimated by the analysis (i.e., differences in size-independent growth coefficients, allocation and cold damage) had little impact on these relationships. Thus, according to our analysis, the observed negative correlations between seed size, RGR, and response to temperature and nutrients result solely from the fact that smaller-seeded species start growth at smaller size.

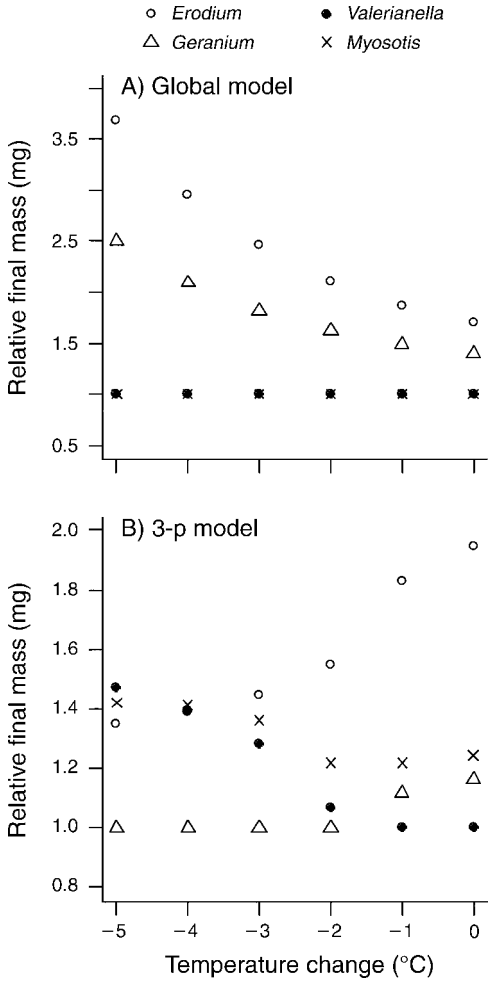

FIG. 6. Predicted final mass (relative to the species with the lowest mass) of the four species with the largest seeds, in simulations where daily temperatures were reduced by a fixed number of degrees each day. (A) The global model predicts no rank reversals, in contrast to (B) the BIC-selected 3-p model. Note that, because *Myosotis* and *Valerianella* have the same biomass at 14 days, they have the same final biomass in the global model where all other parameters are identical.

Predicted response to altered climate

Finally, to determine whether or not species-specific physiology could result in shifts in the biomass ranking of species, we carried out simulations of the global and 3-p models under altered temperature scenarios, where we reduced the daily temperature (ΔT_d) by between 0° and 5°C. In the global model, the four species with the largest seeds did not change rank under altered climate scenarios, but under the 3-p model, *Valerianella*, which had the lowest biomass under the unaltered climate ($\Delta T_d = 0$), had the highest predicted biomass when daily temperatures are reduced by 5°C (Fig. 6). Thus, the estimated differences in species-specific physiology (size-independent growth, cold damage, and allocation) are potentially important in determining the success of different species in different years or in different microclimates.

DISCUSSION

Seed size and RGR

There is a well-established negative correlation between seed size and average RGR (relative growth rate) that has been taken as evidence that small-seeded species are physiologically adapted for rapid growth (Reich et al. 1998, Bloor and Grubb 2003, Shipley 2006). This would help them to successfully exploit a successional niche as they could outgrow larger-seeded competitors given sufficient time (Tilman 1982). We also found the expected strong negative relationship between seed size and average RGR among the nine annual species described here. But this relationship also emerged from the global model in which species share a common growth function, so that the only difference between species is their initial size. Thus, as outlined in the *Introduction* (above), our analysis has demonstrated that a negative relationship between average RGR and seed size can result solely from the decline in instantaneous RGR as plants increase in size. In our experiment, plant growth was best described by a function in which plants grow exponentially at first but then switch to linear growth once some critical mass is reached. That this reference mass was similar for all species, suggests a shared relationship between size and growth across all species (Enquist et al. 1999, Metcalf et al. 2006). Small-seeded species, however, because they begin small, spend longer in exponential growth and therefore have a higher average RGR. But the small-seeded species do not have higher size-independent growth coefficients and are not, therefore, more efficient at fixing carbon. Their absolute growth rates can never exceed that of the large-seeded species, and so they can never "outgrow" the large-seeded species, even given infinite time. While we do not believe that all existing published negative correlations between seed size and RGR are necessarily the product of differences in initial size, we have demonstrated here that these experiments have been inevitably biased in this direction.

There are other interesting consequences of changing the relative time spent in exponential vs. linear growth. Small-seeded species appear to respond more strongly both to fertilization and to an increase in the average daily temperature (inside vs. outside). But again, this occurs even under the global model, in which there are no species-specific parameters in the growth equations. It occurs because environmental conditions, such as nutrient availability and temperature, affect carbon fixation (and hence growth) via a daily multiplier in the growth equation (B_d in Eq. 2). Although in the global model this daily multiplier is the same for all species, its effect depends on the type of growth that the plant is experiencing (whether exponential or linear). And because the proportion of time spent in exponential vs. linear growth depends only on plant size, the effect of

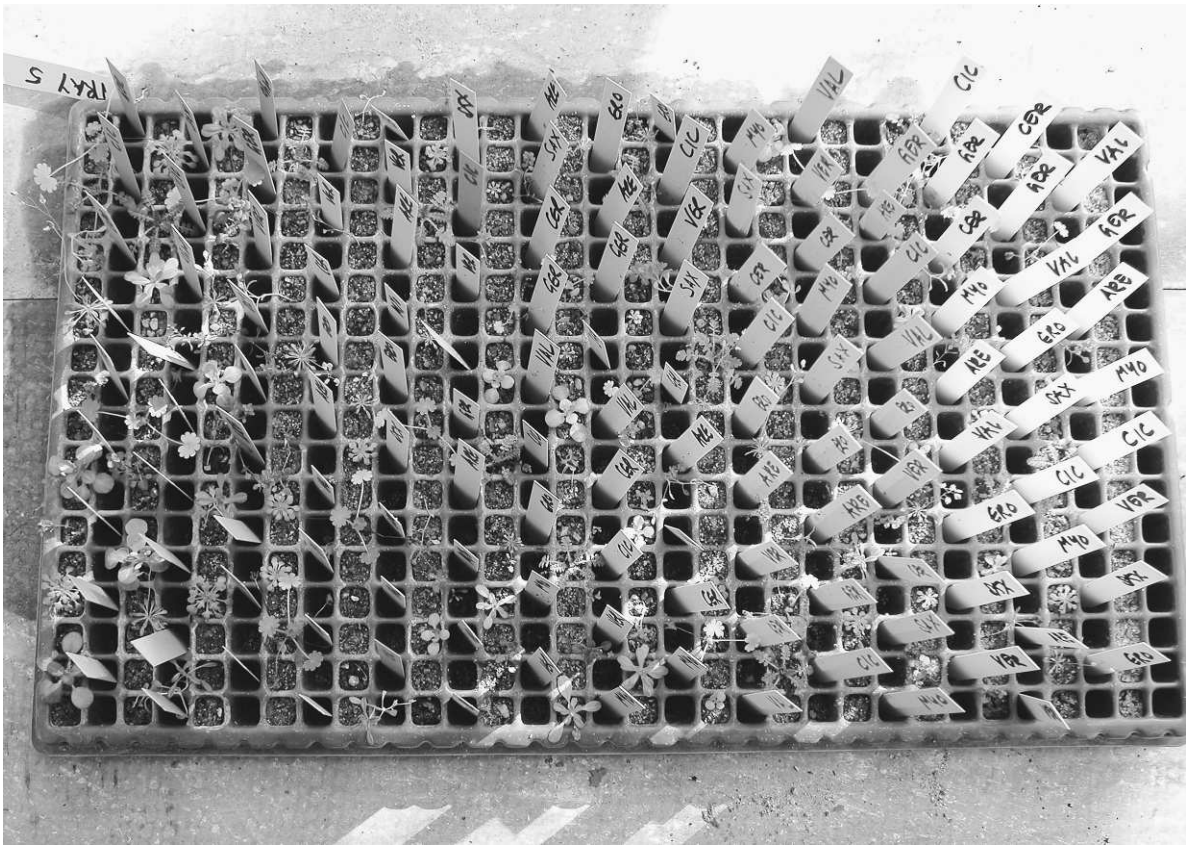

PLATE 1. A tray containing all nine species after 19 weeks of growth. Cells were watered with one of five different dilutions of a complete nutrient solution. Two such trays were harvested at each time interval, one from inside a cool glasshouse and one from outside. Photo credit: Susann Eichenberger-Glintz

the same multiplier is always relatively greater on small-seeded species, which spend relatively longer in exponential growth. Therefore, even in a global model, modifying environmental quality will always have a greater relative effect on the RGR and hence final biomass of small-seeded species. Interestingly, Shipley and Keddy (1988) found that species with the highest RGR under conditions of high nutrient availability showed the greatest reduction in RGR when grown under nutrient-depleted conditions—a result that emerges directly from our simple model without recourse to species-specific physiology (Fig. 5).

Species-specific physiology

The daily growth model presented here disentangles the effects of plant size, environment and species-specific physiology by modeling each component separately. Under, for example, a neutral model, the true species-specific component should be small (Hubbell 2001). However, interestingly, the parameter with the strongest evidence for species-specific differences was the size-independent growth coefficient, *G*. Species appeared to achieve higher size-independent growth coefficients at the cost of increased frost damage, analogous to a growth vs. survival trade-off (Kitajima 1994, Kobe et al. 1995, Sterck et al. 2006). This new and potentially important trade-off was only identified by properly correcting for plant size; otherwise, we would have obtained the paradoxical result that species with the lowest RGR (the large-seeded species) also suffered the greatest cold damage. In contrast to all previous predictions, the correlation between seed size and size-independent growth, as measured by *G*, was positive; that is, *Saxifraga*, despite producing enormous numbers of very small seeds, does not have the growth strategy traditionally associated with an extreme ruderal (Grime 1979)—indeed it has a rather conservative growth strategy, investing in damage protection at the cost of reduced growth.

Although large-seeded species generally grew faster and had lower frost tolerance, there were exceptions. For example, *Valerianella*, a large-seeded species, has an unusually low size-independent growth coefficient and a high degree of frost tolerance. Such differences potentially provide an additional niche axis, orthogonal to that associated with seed size, which could lead to reversals in the success of species in different years (Chesson and Warner 1981, Adler et al. 2006). For

example, simulating a decrease in the average daily temperature of up to 5°C led to changes in the size rankings among the four species with the largest seeds by the end of the growing season (Fig. 6). Such reversals might be an important mechanism for increasing the number of large-seeded species that can potentially coexist (the storage effect: Chesson 1994). However, none of the small-seeded species pursued a high size-independent growth/low frost-tolerance strategy. This is possibly because small-seeded species expect to spend a much longer period in exponential growth and therefore will still be in this phase during the winter months (when frost damage is expected). During the exponential phase, growth is mass dependent, and losing mass during this phase reduces future growth rates, and is consequently much more damaging. Large-seeded species pass the threshold for linear growth at a much earlier stage, well before the winter, and their growth rate is consequently mass independent for much of the winter. Losing biomass during the winter is therefore less damaging as it does not affect future growth.

Community-level consequences

If average RGR does not reveal fundamental physiology, is it still a useful measure? Whatever its physiological underpinnings, the higher average RGR of smaller-seeded species implies a greater return on the carbon investment represented by the seed, and hence greater fitness, measured as annual population growth rate (Cadotte et al. 2006). But, this RGR advantage is only expected to occur where the conditions match those of the experiments, i.e., where each individual seed is given exclusive access to a fixed amount of space, as would happen in an environment of mostly empty patches. At the other extreme, once most patches are colonized we would expect each patch to begin each growing season with a similar mass, rather than number, of seeds (because the final masses of the different species are much more similar than the seed masses; Fig. 6). Under these conditions a more relevant measure of performance might be the average RGR of a given initial seed *mass* per unit area, in the presence of both intra- and interspecific competition. Although competition was not dealt with here, we think that a simple, but mechanistic, size- and growth-based framework similar to the one presented here, might form a useful alternative to current models of annual communities, which tend to assume constant total density, identically sized adults, and lottery competition for microsites—models that are, in fact, extremely difficult to relate to actual plant communities.

Acknowledgments

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APPENDIX A

Species, growing conditions, temperature measurements, and sampling regime for the plant-growth experiment (*Ecological Archives* E089-082-A1).

APPENDIX B

Parameter estimation for the daily growth model (*Ecological Archives* E089-082-A2).

CHAPTER 6

General discussion and conclusion

The diversity of organisms is stunning as illustrated by Wilson (1992) in his book about the diversity of life: “If each of the possible total 100 million of species on Earth were given a page in an encyclopaedia, the volumes would fill six kilometres of shelving”. Along with the species richness, an enormous variation in key life-history traits is observed in plants growing in the same environment. Growth strategies and life-history traits such as seed size vary impressively among species that share the same habitat. Which selection pressures led to such variation in traits? How is this diversity maintained and what are the mechanisms important for coexistence of species? In a changing world, where habitats are disturbed, it is important to preserve diversity and investigate how species respond to increased levels of disturbance. To answer these questions, we used a combination of experimental and modelling approaches to understand coexistence of ecologically similar plant species. We studied traits involved in the competition-colonization trade-off such as seed mass, seed output, flowering time and growth both within species (with *Arabidopsis*) and between species (with a sand dune annual community).

We have investigated the growth and flowering strategies of *Arabidopsis thaliana* and the importance of landscape disturbance on trait diversity with the experiments of this thesis. We explored the impact of different disturbance regimes: static and dynamic landscapes on flowering strategies and seed mass in *Arabidopsis thaliana*. We found that less variation remained in dynamic landscapes for both flowering characteristics and seed mass. Thus, dynamic landscapes exert stronger directional selection on plant traits than the static ones. We also focused on the bolting decision in *Arabidopsis* (the decision to switch from the vegetative to the reproductive phase) and whether plants make the switch at an optimal time. The *Arabidopsis* plants seem to possess the ability to “sense” the environment by initiating bolting when their absolute growth rate can no longer be increased by investing further resources in vegetative structures. Thus, delaying flowering did not lead to higher reproductive mass as traditionally assumed. Our results also showed that on average only 50 % of the total mass

accumulated by a plant occurs before bolting is initiated. The *Arabidopsis* plants are only through half of their active phase. The reproductive part, starting when bolting is initiated, represents the second half of their active phase. Contrary to what stated in previous studies about *Arabidopsis* (Mitchell-Olds 1996), bolting initiation does not represent the end of the plant activity. This important result might change the way to conduct experiments investigating life-history traits, where the analyses were mainly focused on the vegetative phase (Mitchell-Olds 1996, Kroymann et al. 2003).

We investigated two widely used contentious concepts: the seed size/number trade-off and the relationship between seed size and relative growth rate (RGR). We showed in an experiment with *Arabidopsis thaliana* that adult size is only a function of pot size and not of seed size and that a perfect individual-level trade-off emerges between sown seed mass and seed number. Furthermore, we showed that, even within a homogeneous environment, the seed size/number trade-off might not be noticed among individuals, but rather among populations. Thus, it is of primary importance to use models properly comprehended for understanding ecological processes. Failure to do so can lead to false conclusions such as that small-seeded species grow faster in the case of annual plants and therefore compromise the understanding of crucial ecological mechanisms.

During my PhD study, I could not stop thinking “How far are we from reality?” Various difficulties associated with properly measuring something as apparently simple as a growth rate, some questions were raised about the problem of misapplying concepts. Two chapters (chapters 3 and 5) included extensive modelling. First, in the flowering decision study where we modelled total biomass and number of leaves as a function of plant age, and second in the sand dune annuals growth study where a daily growth model was developed. The various methods I used to investigate trait variation during my PhD led me to examine their significance and consequences.

Another inevitable question came: How representative are the studies we perform compare to the real conditions in nature? The *Arabidopsis thaliana* experiments were all performed in controlled conditions in a glasshouse. The sand dune annuals were first grown in an experimental garden and then some plants were transferred to a cool glasshouse to protect plants from frost damage. How realistic are the conditions in a glasshouse?

Field studies vs. glasshouse

Experiments in controlled conditions such as glasshouses or phytotrons have been critized (Granados and Körner 2002, Körner 2006). In a recent article, Körner (2006) explained how given the complex interactions between CO_2 and ecosystems, "…it makes little sense to study CO_2 effects under conditions in which significant covariables are left out (e.g. natural water supply, nutrients, competitors, symbionts etc)". This implies that glasshouse studies might not be relevant to investigate some ecological processes. A glasshouse represents artificial conditions, where there is no wind, no insect, no birds, no fungus or no herbivory. The glasshouse provides uniform and stable conditions which are not realistic. However, Körner himself used glasshouses in previous experiments (Granados and Körner 2002) to investigate the effects of elevated CO_2. He recognized in a study on elevated CO_2 and legumes (Stöcklin and Körner 1999) that the disadvantage of a field study (*in situ*) is that some mechanisms might be obscured by a multitude of unknown interactions. Thus, it would be difficult to detect species responses in the field. In a more recent study (Granados and Körner 2002), the authors decided to stick as closely as possible to realistic conditions, e.g. using soil from the original place of the studied plants (Yucatan Forest, Mexico) although the experiments were performed in growth cabinets in Switzerland. I appreciate his strong will of staying as close as possible to the natural conditions for experiments, but it is quite hard to accept that the results of glasshouse studies are entirely worthless. Most researchers try to do their best to understand biological mechanisms. They are constrained in term of

budget and material supply. Experiments have to be feasible and must contain a reasonable number of replicates and not all laboratories can afford to bring the original soil from the place of origin of their plants. Field studies are of course more natural but there are also disadvantages to conducting experiments in the field. For example, it is not always possible to harvest many individuals from a species in natural conditions (because of protection rules or simply difficulties of finding the number of individuals needed for analysis). Even in the case where enough individuals are found, there is inevitably much more variation among individuals due to the environment. The different individuals may have grown far apart under different environmental conditions and may not be statistically comparable. I agree that using controlled conditions might lead to contentious issues about the significance of the study outcome when it comes to comparing it to natural conditions. On the other hand, I do not think controlled conditions are completely irrelevant. We have to remain aware that an experiment in controlled conditions can be the beginning or the start of investigation of natural processes. They are not necessarily a final step.

Tools of modelling

Another controversial point in ecological studies is modelling. To what extent can we generalize from modelling? Many mathematical tools are available today, but not all biologists are mathematicians or programmers. I was (and still am) quite lost by all the possibilities to perform modelling. It is difficult to start or to express a biological measurement into lines of codes. The concept of the plants can easily be drowned in the numbers and mathematics. Some researchers expressed this as a degradation of species and biology to statistical elements (Sand-Jensen 2007) or being so focused on the model that the plants studied become merely a vague image (Keddy 2005).There is a risk here to drift away from reality, biology and organisms. Some models can even lead to assumptions biologically

unfeasible or unrealistic about plants (Kinzig et al. 1999, Keddy 2005, Turnbull et al. 2008). Non-negligible doubts persist for the application and the use of some models.

However, ecological models are a valuable tool for synthesis but are sources of contentious discussions. Keddy (2005) exposes the weaknesses of theoretical ecology by comparing two types of approaches in plant ecology: the theoretical models and the pragmatical models. The theoretical models provide a broad picture of how plant communities might be organized and how they function, although they explore only the logical consequence of assumptions. The difficulties here are in testing both assumptions and outcomes. The pragmatic models offer a middle approach to the theoretical models and purely descriptive models with only collections of data. For pragmatic models, the patterns come first and the mechanisms come later. The main concern addressed by Keddy (2005) is that it is too easy now to generate large models with complex structures that are extremely difficult to test. He pointed out that: "Too many theoretical models are not falsifiable, and, when data on real plants are carefully gathered to test the models, theoreticians try to trash the data rather than admit the failure of the model". In these cases, the models become more important than the plants or biological reality. Collecting data and building a solid dataset is difficult and time-consuming (even in a controlled glasshouse with *Arabidopsis thaliana*). This should not be underestimated. Many constraints are present to perform experiments. Some theoreticians ask sometimes unrealistic number of replicates. The glasshouse area or simply the work capacity of one person cannot be sufficient to produce such an amount of data. Or they ask unfeasible growth conditions. At the end, when biologists try to carry out experiments, the data collected are never good enough. Hence, no modelling is possible because of the "bad" quality of the data. As Keddy stated (2005): "…pseudo-plant ecologists who prefer the stark simplicity of mathematical models to the dirty reality…" Fortunately, not all theoreticians think like this. This reflects first, the difficulties to communicate and collaborate between theoreticians and biologists, and second the risk of drifting away from reality.

The misunderstanding between ecologists and geneticists

I would like to emphasize the "gap" between researchers belonging to these two different fields. Several chapters from this thesis used the model plant *Arabidopsis thaliana*, which has been used mainly as a model plant for molecular genetics (Somerville and Koornneef 2002, Mitchell-Olds and Schmitt 2006). Recently, an increasing number of ecological and evolutionary studies use *Arabidopsis thaliana* (Shimizu 2002). Lots of reviews about *Arabidopsis thaliana* showed the value of is this model plant for various molecular fields, but few mentioned ecological studies (Somerville and Koornneef 2002). In fact, there are many papers about *Arabidopsis thaliana* ecology, evolution and physiology (Callahan and Pigliucci 2002, Pigliucci 2002, Griffith et al. 2004, Donohue et al. 2005). The model plant *Arabidopsis* has led to many investigations from various fields, but little to interdisciplinary studies combining genetic and ecology. Most of the papers belong to one field, although interdisciplinary research is required and encouraged. Mitchell-Olds (2006) is strongly in favour of new synthesis of functional genomics with evolution and ecology which can benefit each component discipline and thus bring fundamental understanding in biological mechanisms and processes. So why are so few interdisciplinary published studies? This lack of co-operation between ecological and molecular genetic studies could come simply from differences in goals. First, the main goal of the ecologist is to understand relationships between organisms and their environments while the geneticist's main goal is to identify genes and investigate genetic constitution of an individual, group, or class. Obviously, their focuses are quite different. This leads to the second point, in the case of *Arabidopsis thaliana*, ecologists and geneticists use different methods to perform their experiments. Ecologists emphasize the importance of varying environments, while geneticists simply want to grow plants under favourable growth conditions. This is where the misunderstanding begins: in the different ways to study the plants.

Ecologists grow *Arabidopsis thaliana* in various environmental conditions with different nutrient and light levels (Pigliucci et al. 1995, Callahan and Pigliucci 2002), different water regimes (Pigliucci et al. 1995, Pigliucci 2002) to study the plasticity of plants. They wish to understand phenotypic traits plasticity and their ecological significance as plasticity contributes to niche construction and adaptation to new environments (Donohue 2003, Griffith et al. 2004, Donohue et al. 2005). Cultivating *Arabidopsis thaliana* in such standardized conditions as geneticists offer us therefore has been critized. Callahan and Pigliucci (2002) concluded their study on *Arabidopsis* with the remark that "Future ecological studies of A. thaliana […] should also be conducted in field sites or in more controlled experiments where multiple ecological factors vary, and not necessarily in a concordant fashion". This sentence joins in a way the problem of field study vs. glasshouse problem. By standardizing the conditions, we lose the variation of individuals. Some studies even use seeds of *Arabidopsis thaliana* collected from the field to perform experiments (Griffith et al. 2004) because they should be genetically adapted to a particular habitat.

Geneticists generally use seeds coming from many generations of plants cultivated in controlled conditions. Inbred stocks and artificial populations are available for many ecotypes (Mitchell-Olds and Schmitt 2006). The geneticists for example investigate the genes inderlying trait variation. They identify polymorphic genomic regions (quantitative trait loci or QTLs) associated with a particular variation (Mitchell-Olds and Schmitt 2006). As the plants are mostly cultivated in standardized conditions, they study the variation between ecotypes or recombinant inbred lines (RILs) but not the plasticity of traits in different conditions.. QTLs are identified from measured traits of plants grown in a particular environment. They cannot be applied to the plant itself which possibly grows in different environments. QTLs results can be used to understand variation in very specific conditions. The same QTLs may differ if the same plants were cultivated in a different environment. Thus, the question is if the QTLs found in the standardized conditions are really

representative of the plants growing in the field? The standardized conditions are set up to maximize the range of plant phenotypes according to the geneticists. However, although such conditions a maximize growth, are they representative of the natural conditions? Understanding the "rules" plants use to make decisions requires a variety of environmental conditions. To answer these questions, more collaboration is needed between ecologists and geneticists. This has been really emphasized in the paper from Shimizu (2002) with the evocative title "Ecology meets molecular genetics in *Arabidopsis*".

I exposed three issues or difficulties I encountered and found particularly interesting to discuss. Because I had the chance, during my PhD, to work with people from various fields (ecologists, geneticists, and modellers) it broadened my view about how to study plants and the difficulties which can arise as well as the great outcomes with teams of people coming from very different fields. The main challenge would be to speak the same "language". Everyone is specialized in his own field and therefore uses his own vocabulary. It is quite interesting to notice the diversity as well among researchers in this situation. In conclusion, I would like to say that I found the collaborations a success and very enriching. This diversity among researchers is also a source of creativity and essential to scientific research.

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Summary

In this thesis, we investigated the variation in coexisting and ecologically similar species. We analysed traits involved in the competition-colonization trade-off as seed mass, seed output, flowering and growth both intra-species and inter-species.

In **Chapter 2** we investigated the effects of changing the nutrient status of the environment on the nature of the seed size/number trade-off. We used natural genetic variation in the model plant *Arabidopsis thaliana* using a selection of recombinant inbred lines (RILs) showing a large seed size range. In an experiment using two pot sizes we showed that adult size is only a function of pot size and not of seed size and that a perfect individual-level trade-off emerges between sown seed mass and seed number. We also focused on the difficulties to notice the seed size/number trade-off because empirical evidence for this trade-off is limited and contentious; leading some to question the utility of this concept. Here, we showed that even within a homogeneous environment, the seed size/number trade-off might not be noticed among individuals, but rather among populations.

Chapter 3 focused on the bolting decision for annuals (the decision to switch from the vegetative to the reproductive phase) and consider whether plants make the switch at an optimal time. In an experiment using three pot sizes to provide different degrees of belowground growth restrictions, we showed that bolting later did not allow the plant to achieve a higher total mass or accumulate more resources. Leaf production ceased when bolting is initiated, indicating a hard switch from vegetative to reproductive growth. Our results showed that on average only 50 % of the total mass accumulated by a plant occurs before bolting is initiated and rosette growth stops. We demonstrated that on average plants initiate bolting at the inflection point of the logistic growth curve when their absolute growth rate reaches a maximum and thus further investment in vegetative parts would not lead to further increases in growth. The *Arabidopsis* plants thus seem to possess the ability to sense

the environment by initiating bolting when their intrinsic growth can not be maximized anymore. Thus it seems that delaying flowering does not lead to higher reproductive mass when a plant has a fixed amount of resources available in the environment. We did not observe a rigid "clock"-like strategy but rather an optimal solution involving sensing the environmental cues.

Chapter 4 explored the flowering strategy in *Arabidopsis thaliana* in a context of different disturbance regimes: static and dynamic landscapes. We employed a subset of 19 lines from the RILs previously used in chapters 2 and 3 to investigate how landscapes characteristics influenced plant morphological traits associated with dispersal ability through a multi-generational experiment. We measured the effects of five generations of selection on these lines by growing them under standardized conditions. The disturbance regime strongly affected final plant height with plants being taller in dynamic landscapes. However, harvested seed mass, bolting age, flowering age and seed production all experienced similar selection pressures in static and dynamic landscapes, with early flowering being favoured. Although the mean trait values were not different in static and dynamic landscapes, less variation remained in dynamic landscapes for both flowering characteristics and seed mass. This loss of variation was not solely due to the loss of the *erecta* mutation from dynamic landscapes. It is therefore clear that dynamic landscapes exert stronger directional selection on plant traits than the static ones.

Chapter 5 examined the relative growth rate (RGR) concept commonly used to measure and compare species' intrinsic growth potential. Previous studies revealed that small-seeded species have higher RGR, leading to the common belief that small-seeded species grow faster. We showed that, because RGR declines as individual plants grow, it is heavily biased by initial size and does not measure the size-corrected growth potential that determines the outcome of competition in the long term. We investigated the growth of nine coexisting

annual species showing a large range of seed sizes. We developed a daily growth model that included a simple mechanistic representation of aboveground and below ground growth and its dependency on plant size and environmental factors. We parameterized the model using repeated-harvest data from plants of the nine species growing in contrasting nutrient and temperature regimes. In contrast to conventional knowledge, our results showed that large-seeded rather than small-seeded species have higher size-corrected growth potential.

Zusammenfassung

In der vorliegenden Dissertation wurde die Variation in Spezies untersucht, die im demselben Habitat koexistieren. Es wurden Merkmale untersucht, die in das Kompetition-Kolonisations-Verhältnis reflektieren, wie Samenmasse, Samenanzahl, Blühzeit, und Wachstum.

Kapitel 2: In diesem Abschnitt wurde untersucht, welche Auswirkungen verschiedene Nährstoff-Zustände der Umwelt auf die Natur des Samengröße-Samenanzahl Verhältnisses haben. Es wurde sich dazu die natürliche genetische Variation, die in einer Auswahl von rekombinanter Inzuchtlinien des Modellorganismus *Arabidopsis thaliana* vorkommt, in Bezug auf Samengröße zu nutze gemacht. Es konnte experimentell gezeigt werden, dass unter Benutzung zweier unterschiedlicher Topfgrößen die adulte Größe allein von der Topfgröße abhängig ist und nicht von der Samengröße. Daher zeigt sich eine perfekte negative Korrelation zwischen Samengröße und Samenanzahl. Des Weiteren wurde auf die Schwierigkeiten eingegangen, diese Korrelation zu beobachten, gerade im Hinblick auf darauf, dass in der Literatur diese Korrelation kontrovers diskutiert wird. Im Übrigen konnte nachgewiesen werden, das sogar innerhalb homogener Umweltbedingungen die Samengröße-Samenanzahl Korrelation, zwar nicht unbedingt zwischen Individuen, aber zwischen Populationen beobachtet werden konnte.

Kapitel 3: In diesem Kapitel fokussierten sich die Untersuchungen auf die Entscheidungen über Blühiduktion (Entscheidung zum Übergang von vegetativer in reproduktive Phase), im Besonderen, ob Pflanzen bei für die Blüte optimalen Bedingungen die Blühinduktion auslösen. In einem Experiment wurden drei Topfgrößen verwendet, die unterschiedliche Restriktionen für das Untergrundwachstum darstellen, konnte gezeigt werden, dass eine späte Blühinduktion nicht zu einer höheren totalen Biomasse bzw. zu einer

erhöhten Akkumulation von Ressourcen führt. Blattproduktion stoppte, wenn die Blühinduktion einsetzte, was darauf Hinweist, dass der Wechsel zwischen vegetativer und reproduktiver Phase in seinem Charakter eher binär ist. Es konnte gezeigt werden, dass im Mittel nur 50% der gesamten Masse, die von einer Pflanze akkumuliert wird, in der vegetativen Phase produziert wird. Die Untersuchung zeigt, dass im Mittel Pflanzen die Blühinduktion am Wendepunkt der logistischen Wachstumskurve initiieren, wenn die absolute Wachstumsrate ihr Maximum erreicht. Somit würden weitere Investitionen in vegetative Anteile der Pflanze nicht zu einer Zunahme im Gesamtwachstum führen. Es scheint, dass *Arabidopsis* Pflanzen die Fähigkeit innewohnt, die Umweltbedingungen zu erfassen, die dazu führen das das intrinsische Wachstum nicht mehr maximiert werden kann und dann zur Blühinduktion führt. Daher scheint es, das verspätete Blühzeit nicht zu einer höheren reproduktiven Masse führt, wenn die Pflanzen eine fix vorgegebene Menge an Ressourcen in der Umwelt zu Verfügung steht. Es konnte daher keine rigide „Uhr"-ähnliche Strategie des Phasenübergangs beobachtet werden, sondern eher eine die optimalen Umweltbedingungen erfassende.

Kapitel 4: Im vorliegenden Kapitel wurde die Blühstrategie in *Arabidopsis thaliana* unter verschiedenen Bedingungen - statischen und dynamischen Landschaften - untersucht. Dazu wurden 19 der Inzuchtlinien (siehe Kapitel 2 & 3) benutzt, um festzustellen, wie Landschaftseigenschaften pflanzenmorphologische Merkmale, die für Verbreitung von Samen verantwortlich sind, über mehrere Generationen beeinflussen. Es wurden die Auswirkungen an diesen Inzuchtlinien nach fünf Generationen unter Selektion und anschließender Aufzucht unter standardisierten Wachstumsbedingungen untersucht. Pflanzen in dynamischen Landschaften zeigten nach fünf Generationen eine höhere Pflanzengröße, als Pflanzen, die in statischen Landschaften wuchsen. Andererseits erfuhren geerntete Samenmasse, Blühinduktionszeitpunkt, Blühalter, und Samenproduktion ähnliche Selektionsdrücke in

dynamischen und statischen Landschaften, wobei frühe Blühzeiten bevorzugt wurden. Obwohl die Mittelwerte für die einzelnen Merkmale zwischen statischen und dynamischen Landschaften sich nicht unterschieden, konnte eine geringere Variation von Pflanzen aus dynamischen Landschaften für Blüheigenschaftsmerkmale und Samenmasse beobachtet werden. Dieser Verlust an Variation war nicht ausschließlich durch den Verlust der ERRECTA Mutation von Pflanzen aus dynamischen Landschaften zu erklären. Es ist daher anzunehmen, dass dynamische Landschaften eine stärker zielgerichtete Selektion auf Pflanzen ausübt, als statische.

Kapitel 5: In diesem Kapitel wurde die relative Wachstumsrate untersucht, die üblicherweise dazu benutzt wird, um das intrinsische Wachstumspotential von Pflanzen zu bestimmen und zu vergleichen. Vorangegangene Studien haben gezeigt, dass Spezies mit kleinen Samen eine höhere relative Wachstumsrate aufweisen. Das führte zu der Annahme, dass Spezies mit kleinen Samen schneller wachsen. Hier konnte gezeigt werden, dass während die individuelle Pflanze wächst die relative Wachstumsrate abnimmt und somit die relative Wachstumsrate durch die Ausgangsgröße der Pflanze verzerrt wird. Daher misst die relative Wachstumsrate auch nicht das größen-korrigierte Wachstumspotential, dass die Ausgang von langfristiger Kompetition bestimmt. Das Wachstum von neun koexistierender annueller Spezies wurde untersucht und ein hoher Schwankungsbereich an Samengrößen festgestellt. Es wurde ein auf Tage basierendes Wachstumsmodell entwickelt, dass eine einfache mechanistische Darstellung von Übergrund- und Untergrundwachstum und deren Abhängigkeit von der Pflanzengröße und Umweltfaktoren enthält. Dieses Modell wurde durch Biomasse Daten parametrisiert, die aus wiederholter Ernte der neun Spezies stammen, die unter gegensätzlichen Nährstoff und Temperatur Bedingungen gewachsen waren. Im Gegensatz zur allgemeinen Auffassung, wurde gezeigt, dass großsamige Spezies ein höheres größenkorrigiertes Wachstum potential aufweisen als kleinsamige Spezies.

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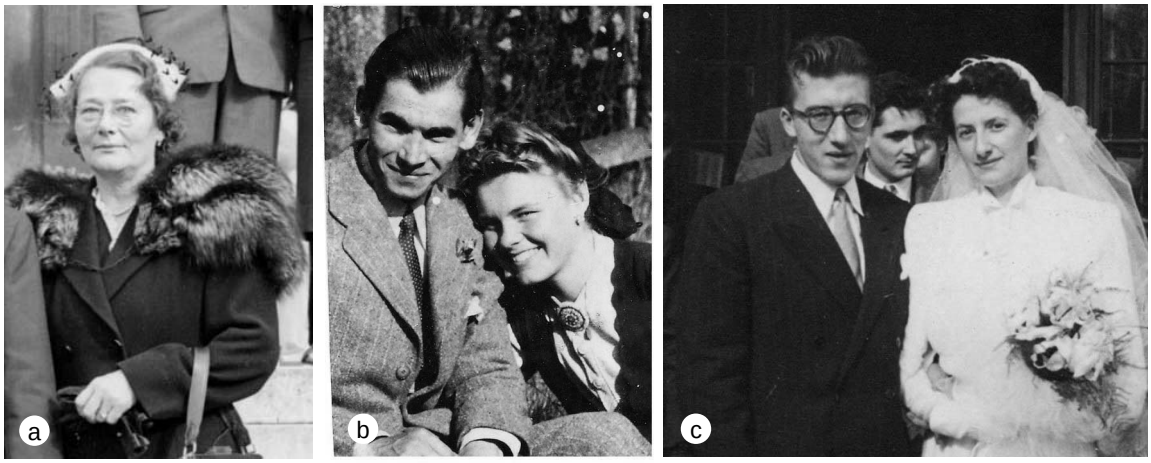
Cloé

Zürich, February 2009.



a) Raymonde Esnault (1891 – 04.04.1992) ; b) Jean (07.09.1926 - 18.12.2008) et Madeleine (21.06.1926 - 23.10.1981) Godineau ; c) Marcelle (29.05.1923 - 17.03.1985) et Guy (03.12.1927 – 07.01.2007) Paul-Victor.

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